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Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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Hybridization Between Ustilago hordei and U. medians. C. C. ALLISON.

Six barley varieties were inoculated with intraspecific and interspecific combinations of monosporidial lines of *Ustilago hordei* and *U. medians*. The pathogenicity of the interspecific combinations was similar to that of one or the other of the intraspecific combinations, except on Peatland barley, on which they produced no smut, whereas the combinations of lines of *U. medians* smutted 9 per cent of the heads. Four combinations were made of F_1 monosporidial lines from a *U. hordei* × *U. medians* cross. All of the resulting F_2 dicaryophytes were distinct in their pathogenicity to the 6 varieties of barley. Two were more pathogenic to Velvet barley than the F_1 dicaryophytes or either of the intraspecific combinations. In the F_1 the head type was intermediate and the chlamydospores were echinulate. In the F_2 there was segregation for compact, loose, and intermediate head type and for smooth and echinulate chlamydospores. Results indicate also that factors for sex, pathogenicity, head type, and chlamydospore-wall characters can be segregated independently.

Black Stele Root Rot of Strawberry. H. W. ANDERSON.

A wilt of the strawberry has caused serious losses in large commercial plantings in Eastern Illinois. During the first bearing season the diseased plants begin to wilt shortly before the first berries ripen. No evidence of the disease has been found during the first growing season. The disease usually appears first in areas where the water-holding capacity of the soil is high, but later may spread to well drained loam soil. The stelar tissues of the roots of the diseased plants are dark brown to black and contain numerous oospores. The cortical tissues may appear normal, but few secondary roots are produced. Wilting results from the fact that the conductive tissues are destroyed. The fungus associated with the disease has been isolated but has not produced conidia in culture or on diseased roots. It is thought to be a Phytophthora. Black stele has been found in widely separated localities in Illinois and neighboring States. It seems to be similar to the Lanarkshire disease of Scotland.

Phytophthora Trunk Canker of Apple. R. C. BAINS.

Phytophthora cactorum has been recognized as the cause of a destructive disease of the trunks of apple trees in Indiana. In 1933 the disease was serious on 12- to 18-year-old Grimes trees. In 6 commercial orchards from 43 to 76 per cent of the trees of this variety were partially or completely girdled. An irregular canker frequently involves the entire trunk and bases of the scaffold branches. The first outward symptom is a wet area appearing on the trunk. Both the bark and sapwood are invaded by the fungus, which in the bark is both inter- and intracellular. *Phytophthora cactorum* has been isolated from 48 diseased trees. The disease has been reproduced on the trunks of 15 Grimes trees of ages 8, 12, and 15 years, following inoculations with mycelium from a pure culture of the fungus. Inoculations made on the limbs resulted in no infection. An 8-year-old Rome tree also was infected by artificial inoculation. No infection was obtained from inoculations made on the trunks of Delicious, Winesap, Jonathan, Liveland, Hiburnal and Virginia Crab trees. Thirteen cankers were treated by scarification with the resulting successful removal of the infected areas from 10 trees.

Importance of Sanitation in Controlling Crown Gall of the Red Raspberry. W. M. BANFIELD and E. C. MANDENBERG.

Mature first-year plants without visible crown-gall infection were selected in the late autumn from fields wherein from 0.1 to 84 per cent of the plants were galled. They were healed in between the rows from several hours to days, then removed, disin-

fected, and stored over winter in virgin sand. Several lots of these apparently clean plants from the 0.1 per cent diseased field were treated as follows: exposed to inoculum or mixed with gall plants and stored in virgin sand, stored in fields habitually used by nurserymen for over-winter storage of bramble-fruit plants, in fields from which diseased plants had recently been removed, or in inoculated sand. All plants were grown throughout the following season in a clean field. Galls developed on 2.6 per cent of the plants taken from the best (0.1 per cent visibly galled plants) field, on 93.7 per cent of those from the worst (84 per cent). Respectively, 40 to 82 per cent of those stored in diseased fields developed gall, 41 to 79 per cent of those held in nursery storage grounds developed gall, 63 per cent of those mixed with galled plants and stored in virgin sand developed gall and 63 per cent of those stored in inoculated sand developed gall.

Tobacco Wildfire Control in Pennsylvania. W. S. BEACH.

Transplanting disease-free tobacco plants to isolated plots that had become contaminated by wildfire a year before shows that *Bacterium tabacum* lives at least a year under field conditions in Pennsylvania and that inoculum from field sources is important in the annual development of the disease. Field observations and seed-bed tests indicate that incompletely decayed butts, stems, etc., of the previous tobacco crop harbor the parasite. In searching for unknown hosts of *Bact. tabacum*, spots characteristic of wildfire, yielding cultures of *Bact. tabacum*, and centering around the flea-beetle injuries, have often been found upon the common weed, *Physalis virginiana*, growing next to wildfire-infected tobacco. This weed is not known to be a host in the absence of tobacco. In experiments on seed beds contaminated with old infected tobacco, it has proved feasible to apply Bordeaux mixture at seed-time with either nonsprouted seed or that sprouted in bulk. Only by this early treatment has it been possible to obtain field plots free of wildfire. In similar experiments, dusting with copper-lime dusts was less effective than Bordeaux mixture. A spray made of calomel and milk powder gave control comparable to Bordeaux mixture, but the calomel was commonly injurious to the tobacco root systems by hindering the development of lateral roots near the soil surface.

A Mosaic on Cabbage in Wisconsin. L. M. BLANK.

Cabbage, *Brassica oleracea* var. *capitata*, plants brought to seed in the greenhouse in the sping of 1934, from heads grown in the field in 1933, showed a high percentage of mosaic. Field observations in August, 1934, established the fact that, in the intensive cabbage section of southeastern Wisconsin, mosaic was generally prevalent on cabbage. Transmission has been successfully accomplished by use of aphids and by plant extract. The symptoms of mosaic on cabbage seed plants in the greenhouse vary from faint to prominent mottling, followed by necrosis and dropping of the lower affected leaves. The effect of mosaic on seed production has not been definitely determined, although some of the affected seed plants produce abundant seed, others little or no seed. Distinct symptoms of mosaic in commercial cabbage fields became increasingly evident during the latter part of the growing season. Plants affected early were usually stunted and retarded in maturity, whereas those affected later in the season were less severely injured. Mottling of leaves was accompanied by an apparent change in the bloom frequently followed by necrosis in local areas on outer leaves and head leaves.

Pathogenicity and Physiology of Pseudopeziza ribis. E. C. BLODGETT.

Anthracnose of *Ribes* spp. and its causal organism *Pseudopeziza ribis* have been investigated by the writer since 1931. Seven isolates of the fungus from Wisconsin, Oregon, Canada, and The Netherlands have been studied comparatively in regard to

morphology, physiology, and pathogenicity. Considerable variation was found in the size and shape of the crescent-shape conidia produced by the isolates in culture and on the host. Microconidia of isolate 2 were over twice the length of those of isolate 4. The optimum temperature for germination of conidia and for growth was about 20° C. Ascospores germinated best at about 12°. Optimum pH of the media used for spore germination and growth was approximately 5.3–7. Infection studies, using conidia (principally) and ascopores, on over 400 plants have shown that: isolates derived from currant are more pathogenic on currant than on gooseberry and *vice versa;* the fungus penetrates directly through either leaf surface; less vigorous hosts are relatively more susceptible; all common garden varieties may become severely infected. The life history of the fungus has been followed in some detail and correlated with epidemiology and control of the disease. The ascigerous stage on currant and gooseberry was found in abundance.

Evidence of the Seed-Borne Nature of Late Blight (Phytophthora infestans) of Tomatoes. O. C. BOYD.

Late blight has not been injurious to tomatoes in Massachusetts since 1905 until the outbreaks of 1932 and 1933. Summer-weather conditions alone seemed not to account for its behavior in 1932 or 1933. Also, there appeared to be no definite relation between its occurrence on potatoes and on tomatoes. An experiment at Amherst in 1934 indicated that *P. infestans* overwintered in tomato seed saved in 1933 and thereby initiated primary infection in the 1934 crop. This occurred both in transplants grown in the greenhouse and in plants from seeds sown in the field at transplanting time. Control plants from healthy seeds did not show primary infection. Assumption regarding the seed-borne origin of the disease was strengthened when it was discovered that diseased seeds from both the 1933 and 1934 crops contained mycelium outside and inside the seed coats and yielded pure cultures of *P. infestans* on agar. Outbreaks of late blight in 1932 and 1933 might perhaps be explained, at least in part, by the more favorable overwintering of the fungus in tomato seeds on farms during the 2 preceding, unusually mild winters. The disease was not important in 1934, in spite of a cool and unusually wet September.

Microorganisms Infecting Pines Attacked by Dendroctonus frontalis. W. C. BRAMBLE and E. C. HOLST.

The southern pine beetle, *Dendroctonus frontalis,* introduces certain microorganisms into the bark of trees that it attacks. These microorganisms invade the sapwood and appear to be a primary factor in the rapid dying of attacked trees. An unnamed basidiomycete, an ascomycete identified as *Ceratostomella pini,* and a new species of yeast, belonging to the Zygosaccharomyces, are carried by the beetle and are the first to invade the sapwood. The basidiomycete penetrates rapidly into the outer growth rings and is abundant during the first week of attacks. Penetration by *C. pini,* and the yeast follows closely on that by the basidiomycete. By the second week they are penetrating as deeply as the latter into the sapwood. During the final stages of the attacks, *C. pini* becomes the most prominent microorganism in the sapwood. Both the basidiomycete and *C. pini* are capable of killing trees, when used for inoculations, provided that the inoculation points encircle the stems of the trees.

Streak, A Virus Disease of Roses. PHILIP BRIERLEY.

A virus disease producing brownish or reddish patterns of ring or vein-banding types in leaves or ring patterns in canes is common in roses at Arlington Experiment Farm, Virginia. Its presence has been disclosed also in the District of Columbia, Beltsville, Maryland, and New York City, N. Y. The understocks Multiflora and Odorata

and varieties of the Tea, Hybrid Tea, Hybrid Perpetual, Hybrid Rugosa, Hybrid Wichuriana, Hybrid Multiflora, Polyantha, Hybrid Bengal, China, and Noisette classes have been found naturally infected. On experimental inoculation by budding, certain hybrid teas, such as Madame Butterfly, develop necrotic areas about the inserted bud. Commonly the stem is girdled at the site of the bud, the distal parts die and the leaves wither but persist. Frequently, nearly black secondary lesions with sharply defined margins, not depressed, appear on young lateral branches below the inserted bud. The virus has been transmitted by tissue union only, not by juice inoculations. Streak is clearly distinct from rose mosaic and apparently distinct from the rose wilt and dieback described by Grieve in Australia.

Symptoms of Rose Mosaic. PHILIP BRIERLEY.

Rose species, understocks, and varieties exhibit a wide range of chlorotic leaf symptoms suggestive of virus infection. Few of these chlorotic types will produce mosaic symptoms on budding to Madame Butterfly or other hybrid teas susceptible to White's rose mosaic. The chlorotic leaf symptoms of White's rose mosaic, proved for various types of roses, scarcely exceed in range those shown by Madame Butterfly alone, namely, local puckering and chlorosis associated with the veins, oakleaf, and etch types, and chlorotic ring-spotting. Among the virus-like types not transmitted to Madame Butterfly by budding are the conspicuous and prevalent crinkle types in Manetti. Symptoms produced in Manetti by budding to White's rose mosaic are less prominent than in Madame Butterfly, but comparable to Briarcliff symptoms. These symptoms in Manetti have thus far been recognized only under glass, and will probably be difficult to identify under field conditions. A yellow variety of mosaic differing from White's rose mosaic chiefly in producing chlorosis of a brighter and lighter yellow has been found in Ontario, New York, Maryland, and Virginia on Hybrid Tea, Hybrid Perpetual and Hybrid Rugosa types. Neither White's mosaic nor the yellow type has been transmitted by methods other than tissue union.

Heterothallism in Peronospora parasitica. HELENA L. G. DE BRUYN.

Pure cultures of the obligate parasite—*Peronospora parasitica*—each derived from a single spore were obtained by inoculating sterile Brassica seedlings on agar slants. Eleven single spores were isolated, originating partially from different places and varieties of cabbage. The difference in pathogenicity or in growth rate was none or only slight, in development of the oospores, however, it was distinct. Three strains were homothallic, forming a great quantity of oospores under suitable conditions. Six behaved unisexually, forming only oospores when grown together with a strain of the opposite thallus group. One strain formed only oospores in a single case, behaving otherwise, as the 6 mentioned above. The 11th strain formed oospores in a small percentage of the tubes when grown either alone or with a strain of the same group, regularly and more abundantly oospores when mixed with a strain of the other group. This strain, therefore, proved to be potentially bisexual, one sex, however, predominating. Of these 8 strains with heterothallic tendencies, 4 belonged to one group and 4 to the opposite group, as proved by intercrossing. As it could not be established in the crosses which mycelium produced the antheridia and which the oogonia, the problem of their sexuality is left undecided.

Damping Off of Alfalfa on Acid and Neutral Iowa Soils. W. F. BUCHHOLTZ.

Pythiaceous fungi have been found especially active on alfalfa seedlings in acid Iowa soils. In 1933 field plots, 41, 48, and 16 per cent were diseased on acid Clarion

loam, Tama silt loam, and Webster silt loam, while on neutral Clarion and Webster silt loam, seven and 6 per cent, respectively, were diseased. In the greenhouse 100 per cent mortality occurred on acid Webster silt loam. The most rapid and general invasion by these soil-borne pathogens has occurred when young emerging seedlings become infected. Lesions on older seedlings were more localized. Steaming or treating with 0.5 per cent formaldehyde either killed or completely inhibited these parasites in acid soil. Limestone, hydrated lime, seed treatment, and soil temperature of 90° C. reduced infection on alfalfa seedlings. Samples of 3 different acid soils were taken at 0 to 2 inches, 2 to 4 inches, 4 to 6 inches, 6 to 8 inches. When planted to alfalfa in the greenhouse 50 seeds per row, the samples in the order named averaged 7, 7, 18, and 39 healthy plants, respectively. Samples of acid Clarion and Tama silt loam, taken in June after two months of dry weather, produced 60 per cent of healthy plants. Greenhouse plantings on similar samples taken in October, after fall rains had kept the soil moist, produced only 12 per cent healthy plants.

Six Microorganisms Pathogenic on Cactus. A BUZZATI-TRAVERSO.

While a student at the University of Milan, I undertook, in cooperation with Dr. L. Pasinetti, a study of the cactus diseases that occurred spontaneously in 2 collections of 300 species. Three types of symptoms were discerned. A general yellowing, turning to reddish brown with a softening of the tissues developed on plants of *Thelocactus nidulans* and *Cactus maxonii*. Specimens of *Coryphantha valida* and *Neomamillaria Gübrowiana* suddenly developed a wet rot that completely destroyed the plants in a few days. A yellow spotting, followed by wrinkling of the epidermis and a blackening and softening of the tissues, occurred in the cambium in plants of *Cereus peruvianus*. Similar symptoms developed on *Cephalocereus senilis* and *Cereus peruvianus*, except that general necrosis resulted. From the first 5 plants named above, I isolated 5 fungi and from the sixth a bacterium. These were all pathogenic and are as follows: *Fusarium cactacearum*, *F. cacti maxonii*, *Sporotrichium cactorium*, *S. traversianum*, *Monosporium cactacearum* and *Bacterium cactivorum*. The 2 species of Fusarium and the bacterium are wound parasties, while the two species of Sporotrichium and the Monosporium were able to enter the host in the absence of a wound.

Growth Association of Microorganisms. J. C. CARTER.

Several fungi and bacteria were grown in association. A study was made of the growth reactions, especially the relative inhibition, of the various associations. Only 2 organisms were grown in association, on potato dextrose agar, in a single Petri dish, at any one time. The fungi that were tested were: *Helminthosporium sativum*, *H. inaequalis*, *Sclerotinia americana*, *Glomerella cingulata*, *Gibberella saubinetti*, *Fusarium moniliforme*, *F. conglutinans*, and *F. lycopersici*. Eleven bacterial organisms were used as inhibitors in addition to *Aspergillus niger* and 2 strains of Penicillium. The fungi named above were grown in association with all the inhibitors listed. Of the 14 inhibitors used, *A. niger* is the only one that produced even a slight inhibitory effect on the growth of *F. moniliforme*. The distance that the fungi were inhibited in most cases was from contact to 7 mm. In general, the Fusaria that were tested were not inhibited readily by the organisms with which they were grown in association. Inhibition of *Sclerotinia americana* occurred more frequently at a distance of 5 to 10 mm. While inhibition of *H. inaequalis* generally occurred at 10 to 15 mm. *H. sativum* was inhibited at a distance of 15 to 25 mm. by 3 bacterial inhibitors and *A. niger*. Of the several fungi tested, *H. sativum* demonstrated the most marked inhibition.

Mechanical Transmission of Two Viruses to Pineapple. WALTER CARTER.

Mosaic of *Commelina nudiflora* in Hawaii is apparently identical with that described from Florida. No natural transmission to pineapple is known. Mechanical transmission by needle puncture has been obtained in a low percentage of trials. Initial symptoms develop on the inoculated leaves, below the points of inoculation. The disease is lethal; the symptoms and their development cannot now be differentiated from those of the yellow-spot virus. A single case of needle transmission of yellow spot from pineapple to pineapple showed the same development of initial spots below the points of inoculation, with later development of symptoms typical of field cases.

The Symbionts of Pseudococcus brevipes in Relation to a Phytotoxis Secretion of the Insect. WALTER CARTER.

Green spotting of pineapple leaves is a toxic effect of feeding by *Pseudococcus brevipes*. It is invariably associated with the presence in the insect of a bacillus-like symbiont. When the insect colony is removed from pineapple to Panicum grass, *Panicum barbinode*, the green-spotting capacity is lost. Concurrently, the bacillus-like symbiont disappears. This process is not complete until the second generation of mealy bugs on Panicum grass emerges. In the interim, intermediate stages in the transition of the bacillus can be recognized. The final and apparently stable form is a minute coccus rod, which is found invariably, but often very sparsely, in the non-green spotting mealy bug. Returning the mealy bug colony to pineapple has thus far failed to result in the reappearance of the bacillus or the ability of the mealy bug to produce green spots. It is concluded that the particular effect of the insect's secretion in causing green spotting is due to the presence of this specific symbiont in the bacillus form.

Serological Evidence in the Study of the Relationships of Certain Plant Viruses, K. S. CHESTER.

The precipitin, neutralization, and complement-fixation techniques have been applied to about 40 types and strains of plant viruses. Twenty-five yellow, white, necrotic, symptomless, and ordinary strains of tobacco-mosaic virus all showed such close serological relationship that they may be considered but minor variants of a single type of virus. Aucuba mosaic of tobacco and Johnson's tobacco virus No. 6 also belong within the same serological group. Cucumber mosaic and tobacco ring spot, both entirely distinct from tobacco mosaic, are readily distinguished from each other by the neutralization test, and each of these comprises strains that are serologically very closely related *intra se*. The latent virus of potato differs serologically from all the viruses mentioned above, and is very closely related to potato ring spot, potato mottle, and British Queen streak, the latter 3 behaving as strains of the latent virus. The vein-banding virus of potato, and the aucuba-mosaic virus of potato, are each entirely distinct from one another and from all of the other viruses mentioned above.

The Development of Root-knot Nematode Galls. J. R. CHRISTIE.

The galls studied were caused by the so-called root-knot nematode, *Heterodera marioni*, and produced by experimentally infesting the radicles of tomato seedlings. Penetration takes place near the root tip and, when permanently located, the head of the larval parasite is usually in the plerome with the body extending into the periblem. During the first 48 to 60 hours, cells of the central cylinder, lying in the region of the parasite's head, remain undifferentiated. After 3 to 4 days the walls of these cells disintegrate and the protoplasmic contents of adjacent cells coalesce forming so-called giant cells. In many instances these cells normally would contribute to the formation

of vessels. Tangential division in the pericycle results in the formation of a layer of small, thin-walled, parenchymatous cells not found in normal roots. Secondary roots, frequently present in the region of a gall, are outgrowths of this layer. Eventually, the innermost parenchymatous cells become differentiated into small xylem elements of irregular shape. These abnormal changes apparently are induced by some secretion expelled through the mouth of the nematode and produced by its oesophageal glands. It is of interest to compare these galls with cecidia and with certain pathological conditions in animal tissues.

A New and Important Factor in the Epidemiology of Tobacco Leaf Diseases. E. E. CLAYTON.

The association of wildfire (*B. tabacum*) epidemics with storms has long been known and explained on the basis of dissemination of the organisms. Recent studies indicate that the major storm effect is in breaking down host resistance. Intercellular leaf spaces become flooded during driving rains, resulting in the appearance of well defined water-soaked areas. The bacteria spread rapidly through such areas and necrotic lesions inches in diameter may be formed in 48 hours. Without the aid of water-soaking, *B. tabacum* usually requires a week to produce lesions ¼ inch in diameter. *B. angulatum* attacks the normal leaf with great difficulty and rarely produces lesions larger than ⅛ inch in diameter. With water-soaked tissues, however, this organism quickly produces the large lesions typical of field black fire. In the field, topping and fertilization practices modify the susceptibility of the plant to both wildfire and black fire. These factors modify leaf resistance to water-soaking. Low topping, and low potash or high nitrogen fertilization, increases leaf susceptibility to water-soaking, and hence to disease. High topping, and high potash or low nitrogen fertilization, have the reverse effect. Other results indicate that attack by certain fungus parasites is also greatly facilitated by leaf water-soaking.

Biochemic Studies on the Metabolism of Crown-gall and Hairy-root Bacteria and on the Composition of Crown Galls. H. A. CONNER, A. J. RIKER, and W. H. PETERSON.

Studies of atypical and pathological multiplication of cells are being made through biochemical examinations of bacterial products and of crown galls. Quantitative determinations showed that the crown-gall bacteria grown in an aerated yeast-infusion liquid medium converted about 2 per cent of the glucose fermented to carbon dioxide and about 10 to 15 per cent to bacterial gum. Hairy-root bacteria converted 20 per cent of the glucose fermented to carbon dioxide and 10 per cent to cells and gum. They formed also small amounts of volatile and nonvolatile acids. These were identified, respectively, as acetic by the melting point of its p-toluidide, and as pyruvic by the melting point of its 2–4 dinitrophenylhydrazone. In addition, both organisms produce large amounts of gum-like materials that have been obtained in the form of compounds with heavy metals. Chemical analyses were made of both preserved and fresh galls on tomato and of fresh raspberry galls. Parallel analyses wree made on contiguous healthy tissue for comparison. The galls were higher than the healthy tissue in ash and total nitrogen but lower in cellulose, starch, pentosans, and sugar. No significant differences were noted in the amounts of alpha amino, amide, ammonia, and nitrate nitrogen, or uronic acids.

Occurrence of Oospores of Peronospora effusa with Commercial Spinach Seed. H. T. COOK.

Oospores of *Peronospora effusa*, the fungus causing downy mildew of spinach, have been found mixed with commercial spinach seed in varying quantities during the last

3 years. The seed examined were obtained from 5 commercial seed firms, were of several varieties, and had been grown in different geographical localities. The degree of infestation varied from no oospore to 1 for every 8½ seeds. No correlation was found between the degree of infestation and the variety or geographical locality where grown. The degree of infestation is probably dependent both on the presence of inoculum and on environmental conditions favoring development of mildew. Large numbers of oospores mixed with commercial spinach seed are a potential source of mildew inoculum. They indicate that mildew was severe in the seed crop and that internal infection of part of the seed may have taken place. The crop grown from heavily infested seed in 1932 was badly damaged by mildew, as was the spinach grown from the same lot of seed planted on new land in 1933.

Spinach Seed Treatments in Virginia. H. T. COOK and J. A. CALLENBACH.

Spinach-seed treatments, tested under commercial field conditions during the last 3 years, have resulted in materially increased stands and yields. Copper sulphate solution, and red oxide of copper, zinc oxide, zinc hydroxide, Vaseo 4 (a zinc fungicide), and Semesan as dusts were used as treatments. Increases as great as 594 per cent in stand and 42 per cent in yield were obtained. Copper sulphate solution, red oxide of copper, zinc oxide, zinc hydroxide, and Vaseo 4 gave approximately equal results, while Semesan treatment was less beneficial. Seed treatment is now practiced on a commercial scale by the local growers and seedsmen, who treat about 90 per cent of the seed used for the early fall crop. Red oxide of copper, which was used at first, has now been replaced to a considerable extent by the zinc materials, which are more uniform physically, more stable chemically, more easily handled, and somewhat lower in cost.

Relation of Insect Injuries and Root Diseases in Sugar Cane. M. T. COOK.

Sugar-cane roots may grow to 6 feet or more in length, but the cortex of all except the last 4 inches dies very early, leading many people to believe that the roots are dead. This dead cortex usually contains many saprophytic fungi. In Puerto Rico three different insects attack sugar-cane roots, the extent of the injuries varying with their location on the roots. It is said by some workers that these injuries become infected by injurious fungi, but the writer's studies indicate that such infection is rare. Some fungi attack the cortex and cause only slight injuries, others are very injurious. Fungi that penetrate into the axis cylinder are most injurious. Thus far the writer has studied the effects of 2 species of Pythium, 1 of Aspergillus, and *Sclerotium rolfsii*.

Relation of Host Vigor to Apple Infection with Xylaria mali. J. S. COOLEY.

Three-year-old apple seedlings, growing at Rosslyn, Virginia, have been inoculated with *Xylaria mali* each month for the past 2 years. The data obtained indicate that susceptibility to infection, size of lesion, and probability of healing are greatly influenced by the state of metabolic activity of the host, which, in turn, is correlated with the season of the year. Evidence indicates that root activity of apple trees varies greatly with the season of the year. The period of greatest susceptibility was associated with the period of least root activity. Suppressed trees, as they grew in the nursery row, were much more susceptible to infection than those growing normally. The fact that suppressed trees showed a much higher percentage of infection during the seasons of slight host susceptibility indicates that the condition of the host as to metabolic activity, rather than other environmental conditions favorable for the fungus, probably is a limiting factor for infection. Trees defoliated in August were much more susceptible to root rot than control trees with leaves. Field observations indicate that a weakened host

condition resulting from drought or other edaphic factors induces greater susceptibility to root-rot infection as it occurs in nature.

Influence of Preceding Crops on Damping Off of Sugar Beets. G. H. COONS and J. E. KOTILA.

Greenhouse experiments with soil from Virginia and Ohio showed that growing sweet clover or alfalfa for 20 to 30 days greatly increased the damping off in sugar-beet plantings that followed. Corn grown for the same period significantly lessened damping off, the basis for comparison in each case being the incidence of damping off in sugar beets occurring in these soils without such preliminary cropping. Beans, as a preceding crop, apparently did not greatly alter initial disease incidence. Using autoclaved soil lightly inoculated (1) with pure cultures of damping-off organisms, or (2) by contamination with unsterilized soil, test beet plantings following sweet clover or alfalfa showed striking increase in damping off; following corn, significant decrease. Such effects did not appear in autoclaved soil that was not inoculated prior to planting preliminary crops. Field evidence has been obtained, in cases where sugar beets immediately followed sweet clover or alfalfa, of the same deleterious influence on stand, in contrast to beneficial effects where corn was the preceding crop. Arrangement of crop rotations to avoid intensification of damping off by alfalfa or the clovers, and to take advantage of its reduction by corn should lead to improved sugar-beet stands.

Some Ecologic Relations of Phytophthora infestans. WILLARD CROSIER and DONALD REDDICK.

The susceptibility of potato plants to attacks of *P. infestans* is not altered by variability of external conditions encountered during the growing period. The most important external factors in the rapid spread of potato late blight are temperature and humidity. A relative humidity of 95 per cent or above must be maintained for about 8 hours to permit the formation of germinable sporangia; and it must persist for a minimum of 11 or 12 hours with liquid water and temperature optima (for germination, swarming, and infection) becoming available at the proper moments in order to permit infection. The spread of late blight over vast areas can not be explained on the basis of aerial migration of the parasite. Infection of new tubers directly from affected seed pieces occurs in sufficient amount to account for the wide prevalence of late blight following a year of no foliage blight.

Scab Resistance in Potato Seedlings. H. M. DARLING, J. G. LEACH, and F. A. KRANTZ.

Of the potato varieties commonly grown in the United States, only those with heavy, russet skins are appreciably resistant to scab (*Actinomyces scabies*). This has led to the popular assumption that resistance to scab is correlated with thick or russet skin. During the past 3 years a large number of seedlings of inbred lines of varied origin have been tested for scab-resistance on heavily infested soil. There is a wide range of resistance among the seedlings tested. A high degree of resistance was found in smooth- and thin-skin seedlings, as well as in the russet types. There was no correlation between color of tuber and scab resistance. Histological studies indicate that resistance is closely associated with the rapidity and efficiency of the formation of normal and wound cork in the lenticels and with the type of cells making up the lenticel structure. Those seedlings having lenticels with smaller and more compact cork cells appear to be more resistant. When certain susceptible seedlings were inbred, both susceptible and resistant seedlings were obtained in the progeny. Likewise, when resistant seedlings were inbred, both susceptible and resistant seedlings were obtained in the progeny. Certain resistant

seedlings, however, yielded seedlings, almost all of which were resistant. A few were slightly more susceptible than the parent. (Divisions of Plant Pathology and Horticulture of the Minnesota Agricultural Experiment Station and the Office of Horticultural Crops and Diseases of the United States Department of Agriculture, cooperating.)

Mycorrhizal and Pseudomycorrhizal Infections of Pine Roots During First Year's Growth. K. D. DOAK and P. L. FISHER.

Determination of the relations involving mycorrhizae in 1-year pine without dissection requires accounting for each portion of the root system and the infections present. Mycorrhizae are distinguished from pseudomycorrhizae by the external mantle that covers the tips, and shows a distinct line of demarcation where it terminates near the mother root. Hypertrophy is evident in true mycorrhizae but lacking or very slight in pseudomycorrhizae. Root hairs are absent from both except for occasional appearances on the noninfected basal portion of the short root. Dichotomy is more frequent in mycorrhizae than in pseudomycorrhizae, even though the latter show slow continued elongation. Rarely short roots show development with both mycorrhizal and pseudomycorrhizal characters; but, when this occurs, each part is separate and distinguishable. Dead mycorrhizae may appear late during the first year's growth, but they are distinguished easily from living mycorrhizae and pseudomycorrhizae. In nurseries of the Allegheny Region, *Pinus strobus, P. taeda, P. echinata* and *P. resinosa* show development of pseudomycorrhizal, as well as mycorrhizal, short roots during first year's growth, but the proportion varies with the nursery. Analyses indicate that the number of each of them is greater than that of noninfected short roots, which appear infrequently.

A Pythium Species of the Megalacanthum Type in Cineraria Roots and the Relation of Putrefaction to Parasitism Among the Pythiaceae. CHARLES DRECHSLER.

Among the Pythiaceae, incapacity for mycelial growth in presence of bacterial contamination necessitates parasitism in living plant structures, or saprophytic invasion of organic materials in advance of competing fungi and attendant bacteria. Such invasion requires rapid mycelial extension. Comparatively slow-growing forms, adversely affected by bacteria, as, *e.g.*, species of Phytophthora generally, are, therefore, constrained in nature to operate mostly as obligate parasites. Rapidly growing forms, similarly affected, including the more familiar species of Pythium, may, however, live also semiparasitically on moribund plant tissues, or saprophytically on tissues newly dead. A few Pythium species—the anomalous *P. anandrum,* and especially *P. mastophorum* and *P. polymastum,* both closely related to *P. megalacanthum*—show greater resistance toward bacteria, producing zoospores in moderately foul water. Large oogonia with basally thick-walled, somewhat mastoid protuberances, characteristic of the *megalacanthum* series, occur in roots of herbaceous plants somewhat more frequently than might be inferred from the paucity of relevant records. Such oogonia were especially abundant in discolored roots of potted Cineraria plants obtained from a greenhouse near Alexandria, Va., April, 1933. The oospores, though normal, failed to germinate, and the fungus was not isolated.

Occurrence of a Species of Aphanomyces on Roots of Spinach and Flax. CHARLES DRECHSLER.

From the discolored roots of somewhat stunted spinach plants collected near Norfolk, Va., Nov. 25, 1932, as also from affected roots of spinach collected in New Jersey, May, 1930, were isolated, in addition to various species of Pythium, many cultures of a species of Aphanomyces. This species has nearly the same dimensions as have been

published for *Aphanomyces cladogamus.* As in the latter, too, the oogonial stalk and the antheridial branch frequently arise close together from the same hypha; and the oogonial stalk rather regularly passes spirally around the antheridial branch. The fungus is, therefore, probably identical with *A. cladogamus;* in any case, it certainly differs from *A. euteiches.* Apparently the same species of Aphanomyces was obtained in numerous cultures derived from discolored roots of stunted flax plants collected in an excessively wet field near Butternut, Wis., July 10, 1931. The discoloration, mostly yellowish and orange, occurred in specks and patches on the surface of the main root. Though the affected portions of the cortex were noticeably softer than the healthy portions, no general decay of the root was present at the time of collection.

Thermal Inactivation of Some Tobacco Viruses: Standardization, Technique, and Illustrative Data. B. M. DUGGAR.

The temperature lag involved in the determination of thermal inactivation periods was followed with extracted juices of diseased plants, using as containers for the extracts test tubes of medium (culture) and small (serological) size in contrast with standardized, thin-walled, capillary glass tubes. In addition to the thermal bath the apparatus consists of a thermocouple in conjunction with a potentiometer and a galvanometer setup, also suitable stirring devices in the case of the tubes. Readings were made every 15 seconds. A significant lag period was found with the tube methods but no measurable lag with the capillary methods. Accordingly, the capillary method is recommended as a standard technique in precise work on thermal inactivation. Data are presented showing the inactivation percentages or survivor values for the virus of typical tobacco mosaics and for the virus of tobacco ring spot. A direct leaf-blade-immersion method also has been used, and with these viruses the technique is comparable to the capillary method. Owing to differences in tissue texture in other host plants, the tissue method is not regarded as having such general applicability.

Some Factors Affecting "Longevity" in Vitro of Viruses of Tobacco Mosaic and of Tobacco Ring Spot. B. M. DUGGAR and D. F. MCALISTER.

The survivor value of the virus of typical tobacco mosaic in tobacco extract is favorably affected (1) by condensation of the extract at low pressure and temperature; likewise (2) by the addition of low concentrations (antiseptic) of ethyl alcohol. The active period of the virus of tobacco ring spot (Wingard strain) also is prolonged, within limits, by low concentration of alcohol and by the addition of certain buffers at definite pH levels, notably around pH 5.8–7.0, phosphate buffers being more favorable than citrate. The dispersion of the ring-spot virus also is enhanced by the phosphate buffer, as measured by incidence of primary infection lesions.

Sand Culture of Seedlings as a Damping-off Control. A. A. DUNLAP.

Certain species of seedlings that have shown tendencies toward damping-off in soil have been grown successfully in pure sand, with mineral nutrients added in solution. Both preemergence and postemergence damping-off have been much less in the sand than in nontreated soil. The results of growing seedlings in sand, have compared favorably with those obtained from the use of the usual seed or soil treatments.

A Neglected Factor in the Planning and Interpretation of Fungicidal Tests. H. W. DYE.

Practically all comparative fungicidal tests on apples are applied according to a schedule developed over a period of years through the use of lime-sulphur solution, and

are based on the minimum number of applications with a material that, because of its nature, retards normal development to a varying extent. In comparative tests with fungicides that allow a more normal growth, the advantage in control is always with the material that permits the least foliage and fruit development between applications, even though all may be of equal fungicidal value. Is it not possible that this simple difference in the growth factor with different fungicides, which is often overlooked, may explain some of the differences that occur in comparative fungicidal tests, and is it not worthy of more serious consideration in planning such tests and in interpreting their results?

Soil Treatment with Sulphur and Limestone for Control of Bacterial Wilt of Potatoes. A. H. EDDINS.

Inoculated sulphur applied to the soil at the rates of 200, 400, 600, 800 and 1,200 pounds per acre decreased the percentage of bacterial wilt (*Bacterium solanacearum*) infection of potatoes in Florida. Control was correlated with the amount of sulphur applied, as the disease disappeared most rapidly and most completely from plots receiving the greatest amounts where the pH of the soil was reduced to 4.50 and lower. During 5 seasons the most beneficial rate at LaCrosse, Florida, was 800 pounds per acre and during 2 seasons at West Tocoi, 600 pounds per acre. Smaller quantities were less effective, while larger applications reduced the yields of potatoes. Agricultural limestone applied in January, 1934, at LaCrosse, at the rate of 2,000 pounds per acre to plots treated with 1,200 pounds of inoculated sulphur per acre in 1929 increased the yield of healthy marketable tubers 12.7 barrels per acre, as compared to the yield of plots receiving neither of these agents; tuber infection in the treated plots was 0.3 per cent and in the check plots 2.6 per cent. A like amount of limestone applied in November, 1933, at West Tocoi, to plots treated with 800 pounds of inoculated sulphur per acre in October, 1932, increased the yield 17.1 barrels per acre; tuber infection in the treated soil was 0.4 per cent and in untreated soil 25.7 per cent.

Field Trials of Pentachlorethane, Tetrachlorethane, and Xylol as Affecting Phymatotrichum Root Rot and Host Plants. WALTER N. EZEKIEL and J. J. TAUBENHAUS.

Pentachlorethane, tetrachlorethane, and industrial xylol were applied in the soil around cotton plants and chinaberry, maple, and elm trees with *Phymatotrichum omnivorum* root rot, in holes 6×6 inches apart and 6 inches deep, at rates calculated on the soil weight usually to 4 feet deep. Roots were excavated and cultured to determine viability of the fungus. At 500 and 1,000 ppm., the materials killed *P. omnivorum* on and within these roots; 250 ppm. was not uniformly effective. Whether the fungus is completely eradicated from the soil in treated areas remains to be determined. Applied around normal trees or shrubs left undisturbed after treatment, tetrachlorethane at 500 or 1,000 ppm. caused rapid, severe injury to these plants, xylol slower injury, and pentachlorethane still slower and less severe injury. In some other plats, materials were applied to the soil at a higher rate prior to planting cotton. Only scattered stunted plants were obtained from plantings made even 3 months after the tetrachlorethane treatment; while a good stand was secured from a planting 16 days after the xylol treatment.

Infection of Apple Leaves by Physalospora cydoniae. H. H. FOSTER.

Leaves of potted apple trees were inoculated by spraying with pycnospores of 30 isolates from different regions. Following inoculation, the trees were held in a moist chamber for 24 hours at 20° C., and incubated in the greenhouse. Only 10 of the 30

isolates gave consistent infection. The fungus was reisolated at will. Eight of the 10 leaf-infecting isolates were secured from rotted apple and quince fruits from eastern States, one was obtained from a Chinese quince fruit from France, and one from an apple leaf from South Carolina. Isolates obtained from Wisconsin and certain nighboring States induced little or no infection. Some isolates induced visible lesions within 24 hours; others only after 48 to 72 hours. Penetration was found to take place through the stomata. In 2 trials 21 varieties of apple trees were tested for comparative susceptibility of leaves to *Physalospora cydoniae*. Two isolates, each inducing infection, but differing in certain respects, were used in these trials. Some indication of differences in varietal susceptibility was apparent.

Spotted Wilt of Truck Crops and Ornamental Plants. M. W. GARDNER, C. M. TOMKINS, and O. C. WHIPPLE.

The host range of the virus of the spotted wilt of tomatoes in California, as indicated by inoculation with the virus in the green house or recovery of the virus from naturally infected plants, includes, in addition to the hosts reported in previous abstracts, cauliflower, celery, *Nicotiana glauca*, and species of Amaryllis, Begonia, Browallia, Campanula, Cheiranthus, Delphinium, Emilia, Gloxinia, Godetia, Gaillardia, Layia, Papaver, Pentstemon, Primula, Salvia, and Verbena. The virus has been transmitted with *Thrips tabaci* from Emilia to Emilia and tomato, and from calla to tomato, and with *Frankliniella* sp. from Datura to poppy and from tomato to tobacco. The virus has been found to survive 73 hours' storage at 0° C. The disease is most prevalent and severe in the cooler coastal districts, particularly where truck crops or ornamentals are grown more or less all the year round. Relatively low temperatures seem to favor the disease.

Availability of Copper in Bordeaux Mixture Residues and Its Absorption by Conidia of Sclerotinia fructicola. M. C. GOLDSWORTHY and E. L. GREEN.

The available copper in 2–4–50 Bordeaux-mixture residues that had been subjected to aging and weathering and that absorbed within the conidia and germ tubes of *S. fructicola* were determined by the use of nitroso-chromotropic acid. The available copper, inherent in such residues and amounting to at least four ppm. by volume of the original mixture, was lethal to germinating and germinated conidia. Smaller amounts of available copper were found to be inhibitory to the growth of the conidia. With inactive conidia, intake of copper was relatively slow, but with actively germinated conidia, it was very rapid. The intake of available copper was not affected by conidial secretions or metabolic by-products. Intake of copper was associated with intake of other ions. Germinating conidia do not have the power to restore the supply from the remaining insoluble residue, once the available copper is washed away by rains. Restoration of the available copper is apparently brought about by weathering.

Control of Aster Leaf Rust. C. E. F. GUTERMAN.

Leaf rust (*Coleosporium solidaginis*) is very prevalent in commercial plantings of the China aster in New York State. During the 1934 growing season, several sprays and dusts were tested under field conditions in an attempt to obtain control of the disease. The following fungicides, listed in the order of their efficiency, were used: Sulfodust, Green Kolodust, Flotation sulphur spray and 3–1½–50 Bordeaux mixture. Due to abundant rainfall and an unusual amount of rust, 8 applications were necessary to provide adequate protection. With the check plots averaging 18.9 lesions per leaf, a reduction to 0.2 lesions per leaf was obtained with the Sulfodust. Marked differences in

varietal susceptibility to rust were observed in tests involving 60 commercial varieties of the China aster. Under the conditions of these experiments, the varieties California Giant, white; Giant Branching Comet; Salmon Queen; Imbricated Pompon, blood red; Lilliput flowered, rose; Lilliput flowered, dark blue; and Lilliput flowered, white, proved to be essentially immune.

A Liquid Formaldehyde Treatment to Control Damping Off of Flower Seedlings. C. E. F. GUTERMAN and L. M. MASSEY.

Six per cent formaldehyde dust used at the rate of 1½ oz. per square foot for the average-size flat is a common method now in use for damping-off control. When used in proper amounts, a 40 per cent formaldehyde solution has given results equally as good and often better. Using a soil mixture consisting of half top soil and half sand in flats measuring $18\frac{1}{2} \times 14 \times 3$ inches, it has been determined that $12\frac{3}{4}$ cc. of the formaldehyde solution when diluted 5 or 6 times its volume with water and thoroughly mixed with the soil will give excellent results. At this streigth, a 12–24 hour wait is necessary after the treatment before it is safe to plant most seeds. Six to 9 cc. may be used for the same amount of soil and the seeds planted at once. As is true with formaldehyde dust, the flats must be watered thoroughly after sowing the seed. Among the ornamentals that respond well to this treatment are Calendula, China aster, Clarkia, Gypsophila, Kochia, larkspur, Lunaria, marigold, Scabiosa, stock, strawflower, and sweet pea. Anchusa, Campanula, and snapdragon gave irregular results due to injury. The advantages of the method are its ease of application, economy, and assurance of uniform strength.

The Effect of Mosaic on Transpiration of the Bean. A. L. HARRISON.

The effect of mosaic on transpiration of Stringless Green Pod Refugee beans, *Phaseolus vulgaris*, was determined by several methods; namely, auto-irrigators (Livingston and porous-pot), balances, and cobalt chloride papers. The rate of transpiration was significantly less per unit of surface area and per unit of dry weight in mosaic than in healthy plants. Leaves with primary symptoms transpire much slower than leaves with secondary symptoms, as measured by cobalt chloride papers. The same method revealed that the light green areas of mosaic leaves transpired significantly less than the dark green areas. Mosaic did not effect the rate of transpiration on leaves formed before infection. Leaf areas were determined by multiplying the product of the width and length of the leaves by the conversion factor for the leaf or leaflet in question. Detached pods from mosaic plants transpire faster per unit of surface area and per unit of fresh weight than detached pods of the same size from healthy plants. Size of pod has a greater influence than age on the rate of transpiration. Small pods transpire faster per unit of surface area and per unit of fresh weight than large pods of the same age. (Cooperative investigation between the New York (Geneva) and the Cornell University Agricultural Experiment Stations.)

Vascular Disease in Poplar and Willow. CARL HARTLEY and B. S. CRANDALL.

There occurs in the District of Columbia a stain in the wood of Lombardy poplar, *Populus nigra italica*, at first water-soaked but later changing to brown, commonly with an intermediate red stage. It was noted by L. W. R. Jackson some years ago in dying trees, and is now found to be of very common occurrence in apparently healthy trees. It does not cause death until it has occupied almost the entire cross section of the trunk. Short rod bacteria, forming quantities of gas, are readily isolated from wood near the margin of the discoloration. Observation indicates this vascular disease to be one of the

principal causes of the short life of the Lombardy poplar in the neighborhood of Washington. A somewhat similar condition is frequent in cottonwood, *P. deltoides;* goat willow, *Salix caprea;* and some other willows not yet identified. In both poplar and willow the symptoms in some respects suggest the water-mark disease of willow in Europe. Reddish discolorations containing bacteria also occur in numerous young plane trees, *Platanus* sp., in a nursery near Washington. (Bureau of Plant Industry and Emergency Conservation Work.)

Control of Downy Mildew of Tobacco. R. G. HENDERSON.

A number of fungicides have been tested under greenhouse conditions for the control of downy mildew of tobacco. Some of the materials included in these experiments are: Benzoic acid, cuprous oxide, coposil, copper phosphate, acetic acid, and copper-molasses mixture. Of these, benzoic acid, cuprous oxide, and copper-molasses mixture were the most effective. Cuprous oxide delayed the development of the disease several days, but under conditions most favorable for the disease, this spray did not prevent it. Benzoic acid was more effective than cuprous oxide and should be tested further. The copper-molasses mixture was very effective in controlling mildew, although, in these tests some mildew developed on seedlings sprayed with this material. As reported last year, calcium sulphide has given very good control of downy mildew. Under greenhouse conditions the growth of plants sprayed with calcium sulphide was very much retarded, but in experiments conducted last spring in outside beds the plants did not show this stunting effect. In the greenhouse experiments, calcium sulphide applied at 3-day intervals gave better control of mildew than the copper-molasses mixture. Several other chemicals in addition to those mentioned above are now being tested.

Studies on the Soft Rot and Colon-Typhoid-Dysentery Groups of Bacteria. 1. Pathogenicity. W. D. HENRY.

One hundred and twenty organisms belonging to either the soft-rot or colon-typhoid-dysentery group of bacteria have been used in repeated attempts to study the variability in pathogenicity of these organisms. Carrot varieties, Oxheart, Chantenay, Long Orange, and Danvers Half Long were used as the chief test host but one or more tests were made on cabbage, potato, iris bulbs, delphinium, radish, turnip, lettuce, banana, parsnip, and canna. Aseptically cut slices from sterilized carrots were placed in sterile moist Petri dishes. Inoculation was made by placing one loopful of culture in Bacto-beef broth on the freshly cut surface of carrot slices. Results obtained showed considerable differences in pathogenicity of organisms causing rot on carrots of different varieties and also on carrots of the same variety. Sixty-six out of these 120 organisms rotted Oxheart variety carrots. However, not all of these proved consistently pathogenic. The first series of inoculations showed that 47 organisms caused rot, 46 proved pathogenic in the second trial, only 24 in the third and 17 in the fourth. The inoculation experiments extended over 10 months. Some organisms retained their pathogenicity in all tests, while others lost and regained it without any regularity. Additional trials with other varieties of carrots gave similar results.

Apothecium Production in Sclerotinia trifoliorum and S. sclerotiorum. LAWRENCE HENSON.

In a study of crown rot of red clover at Lexington, Kentucky, apothecia of two species of Sclerotinia were found in large numbers—one (*S. trifoliorum*) in October, the other (*S. sclerotiorum*) in May. A satisfactory technique for production of apothecia consists in planting sclerotia on plain 1 per cent agar slants in 2×8.5 cm. vials plugged

with cotton. Apothecia developed in vials out of doors simultaneously with those of the same strains planted in soil. *S. sclerotiorum* developed stipes in the icebox at 48 to 51° F. in 50 to 60 days, and *S. trifoliorum* in 25 to 30 days. When transferred to light, both species matured normal apothecia. Of 63 cultures of *S. trifoliorum* placed out of doors in the fall, 51 matured apothecia; but 17 cultures of *S. sclerotiorum,* set out on September 27, failed to do so. In day-to-day plantings out of doors, some stipes developed in 14 days, the average temperature being 64.5° F. The production of functional apothecia by 34 single-spore cultures of *S. trifoliorum* from 7 apothecia, and by 3 single-spore cultures of *S. sclerotiorum,* 9 single-spore cultures of which again developed functional apothecia, demonstrates that at least some strains of these species are homothallic. (Cooperative investigations betweeen the Division of Forage Crops and Diseases, Bureau of Plant Industry, Department of Agriculture, and the Kentucky Agricultural Experiment Station.)

Longevity of the Fire-blight Organism in the Honeybee Environment. E. M. HILDEBRAND.

The longevity of the fire-blight organism, *Erwinia amylovora,* when introduced into the honeybee environment has an important bearing on the question of whether the honeybee is capable of overwintering the bacteria. Studies conducted in New York over a period of 3 years show that the probability of the bacteria overwintering in the beehive, when introduced through the normal channel of food, is remote, because of: (1) The disappearance within 3 days of the bacteria from honey elaborated from food containing the organisms; (2) the inability to identify the organism with stages of the life cycle of the insect; (3) the relatively high temperatures (up to 35° C.) of the bodies of honeybees and of the interiors of hives; (4) the shortness of life of the bacteria held in sugar solutions at different concentrations and constant temperatures; and (5) the relatively poor dissemination of the bacteria to flowers when caged with honeybees that had been fed heavily infested sugar solutions. The same colonies of bees were used throughout the series of tests without apparent injury. The validity of the conclusions of these studies, a detailed report of which will be published, receive support from studies conducted elsewhere on the shortness of life of other bacteria in honey.

Modes of Entry of Erwinia amylovora into the Flowers of the Principal Pome Fruits. E. M. HILDEBRAND and L. H. MACDANIELS.

Blossom-blight control with bactericides has been attempted, as blossom time is a critical period in the life history of the fire-blight organism. The results of studies begun in 1932 were variable and sectioning of the flowers revealed a different picture from that in the literature with respect to the natural openings. In view of this situation the need was apparent for an understanding of the normal and pathological anatomy of the flowers, and such studies were undertaken with station coworkers. The bacteria apparently enter the flowers of pear, apple, and quince only through natural, or artificial, openings. The types of natural openings encountered were: (1) Noncutinized tissue (stigmatic surface, anther); (2) hydathodes (sepals); (3) stomata (style, fruit exterior, sepals); and (4) specialized nectar-secreting stomata (receptacle cup). The distribution of the natural openings was such that they functioned readily as infection courts to the bacteria, whether the agency of dissemination was rain or insects. The floral organs varied in their relative susceptibility to invasion. When atomized with bacterial suspensions, the receptacle cup of pear and the stigmas of apple and quince were frequently invaded. The remoter avenues of entry, as petals and anthers, sometimes resulted in blossom infection when they alone were inoculated.

Some Fungicidal Possibilities of Red Copper Oxide. JAMES G. HORSFALL and JAMES M. HAMILTON.

This is a progress report dealing with fungicidal qualities of red copper (cuprous) oxide, based upon data obtained, using the material as a seed disinfectant and protectant and as a foliage spray and dust, in comparison with standard fungicides. (1) It gave satisfactory control of seed decay, oat smut, wheat bunt, apple scab, tomato early blight, rose mildew and black spot, and snowberry anthracnose. (2) It sometimes gave typical injury on copper-sensitive plants, but seemed as generally safe as Bordeaux. (3) Lime and cottonseed-oil emulsion reduced the injury without curtailing effectiveness. (4) It did not stunt hop cones nor distort succulent tomato and rose leaves when Bordeaux did. (5) It adhered strongly to seeds and foliage and persisted through rains because it is highly insoluble, has a small particle size, and probably bears a positive electric charge. (6) The high weight/volume ratio and small particle size suggest that it can be dusted with greater than usual velocity. (7) High copper content of the molecule almost eliminated objectionable spray residue. (8) The cuprous ion purchasable cheaply in cuprous oxide seemed more toxic than the cupric ion widely used in plant pathology. (9) It seems compatible with usual insecticides.

Inoculation Tests with Phytomonas stewarti and P. vasculara. S. S. IVANOFF.

Greenhouse and field inoculations on sorghum, *Holcus sorghum*, Sudan grass, *Holcus sudanensis*, and yellow foxtail, *Setaria glauca*, with strains of *Phytomonas stewarti* induced symptoms resembling those caused by the same pathogen on corn. The degree of similarity depended on the host. Young sorghum plants in the greenhouse, inoculated at the base by punctures, developed red stripes along the leaf veins. The shape and development of these stripes resembled those induced on corn. Field inoculations on sorghum induced reddening and plugging of the vascular bundles, with occasional formation of pith cavities. Red leaf stripes, similar to those produced on sorghum in the greenhouse, were produced on Sudan grass in the field. The stripes occurring on the leaves of yellow foxtail were narrow and tan colored. In the greenhouse, sweet corn plants, when inoculated at the base with *P. vasculara*, developed symptoms similar to those induced in the same host by *P. stewarti*, such as water-soaked stripes upon the leaves, stalk rot, and general stunting. Puncture inoculations of sorghum plants with the sugar-cane organism induced leaf symptoms resembling those induced by the same pathogen on sugar cane, *Saccharum officinarum*, and by *P. stewarti* on sorghum.

Resistance of Sweet Corn to Bacterial Wilt. S. S. IVANOFF and A. J. RIKER.

Studies on resistance of sweet corn to bacterial wilt (*Phytomonas stewarti*) were conducted during the summers of 1933 and 1934. Resistance was tested both by artificially induced and natural infection of more than 200,000 plants, including approximately 2,000 inbreds and hybrids secured from Golden Bantam. The size of the experiment and the uniformity and efficiency of introducing 5 wilt-inducing strains of the organism into the plants was greatly increased by using a plant inoculator. Although no immune plants were found, considerable differences in susceptibility were observed among the strains. In the same inbred strain, acquired vigor, because of better growing conditions, appeared directly correlated with increased resistance. Apparently, hybrid vigor is responsible for some resistance. At least 3 other factors commonly appeared correlated with resistance: inherent vigor, time of maturation, and "true resistance." Usually, late strains were more resistant than early ones. True resistance was found in vigorous as well as in weak, in late as well as in early strains. After consideration of factors mentioned above, it appeared commonly that crosses between susceptible and

susceptible inbreds gave susceptible hybrids; between susceptible and resistant inbreds gave resistant hybrids; and between resistant and resistant inbreds gave resistant hybrids.

A Phytophthora Root and Collar Rot of Pinus resinosa Seedlings. L. W. R. JACKSON and B. S. CRANDALL.

The red pine, *Pinus resinosa*, seedling and transplant stock in one of the eastern forest nurseries has suffered heavy losses for several years from a detructive type of root and collar rot. The disease is characterized by a dry type of bark rot and a vascular infection that causes the wood to become dark colored and very resinous. Large pitch pockets in the wood are common. The infection involves the entire root system and may extend upward several inches in the stem. Isolations from root and collar infections consistently yielded a fairly high percentage of cultures of a Phytophthora closely resembling *P. cinnamomi*. In artificial inoculations of potted red pine plants, the fungus produced disease symptoms similar to those occurring in nature. By means of various kinds of artificial inoculations the fungus has been found to be the causal organism of the root and collar rot of red pine. Other fungi found associated with the root and collar rot lesions were *Pestalozzia* sp., *Sphaeropsis* sp., *Sclerotium* sp., and *Rhizoctonia* sp. All of these fungi when tried in artificial inoculations were capable of causing resiniferous lesions, and sometimes death, but failed to reproduce the typical symptoms of root and collar rot. (Bureau of Plant Industry and Emergency Conservation Work.)

A New Disease Affecting Platanus orientalis in the Eastern United States. L. W. R. JACKSON and BAILEY SLEETH.

Oriental planes in one community in Delaware County, Pa., have been dying at an alarming rate for several years. Within a year or two the foliage of the afflicted tree becomes exceedingly sparse and then suddenly wilts. The bark on the main trunk is covered with numerous longitudinal lesions, which are associated with black wood discolorations that may extend inward to the pith. Isolations from the discolored wood have consistently yielded pure cultures of an endoconidiophora form of Ceratostomella. In culture it gives off a strong banana-oil odor. Fourteen 2-year-old cuttings of oriental plane, in greenhouse pots, were inoculated and all of them wilted after 4 to 13 weeks. Wilting occurred only on parts above the point of inoculation. The fungus produced a black vascular discoloration in the stem of the inoculated cuttings, for a distance up to 6 inches, which was identical with the infections in nature. The reisolations yielded pure cultures of the fungus in almost every case. Inoculations without wounding produced, in 11 days, bark lesions ½ inch long. Inoculations on 2-year-old seedlings of *Ulmus americana, Quercus palustris,* and *Q. borealis* failed to produce infections. No inoculations were made on *Platanus occidentalis*. (Bureau of Plant Industry and Emergency Conservation Work.)

The Value of Zinc Sulphate as a Peach Spray Ingredient. K. J. KADOW and H. W. ANDERSON.

Experiments have been conducted in Illinois (1929 to 1934) to determine, if possible, the value of zinc sulphate as a fungicide and bactericide, as well as its relation to peach growth and spray injury. The results indicate that zinc sulphate added to lime or lead arsenate-lime spray does not improve their fungicidal or bactericidal value. Lime alone is more effective than zinc sulphate. Zinc sulphate improved the growth of peach seedlings grown in sand and maintained by a nutrient solution, but had no noticeable

effect on seedlings grown in black silt-loam. No growth improvement has been observed when zinc sulphate-lime or zinc sulphate-lead-lime sprays were applied to the peach under Illinois field conditions. Field records and chemical analyses show conclusively that zinc sulphate added to lead arsenate-lime sprays almost eliminates water-soluble arsenic from the mixture. The chemistry of this reaction is not fully understood. Apparently its action may be two-fold: the prevention of rapid carbonation of lime and precipitation of water-soluble arsenic as zinc arsenate whenever it is formed. When lead arsenate-lime sprays are applied to the peach in Illinois zinc should always be added to prevent spray injury. Zinc sulphate has no commercial value when applied specifically as a fungicide, bactericide, or plant stimulant.

The Production of Asexual Spores by Pleurotus corticatus. FRANK KAUFERT.

In 1931 an agaric, tentatively identified as *Pleurotus corticatus,* was isolated from fire-scarred and decayed red gum and oak in the Mississippi Delta of Louisiana and Mississippi. Large, black-top coremia developed on the pileus, gills, and stipe of a fruiting body found in nature. They were found also on mycelium in culture and on fruiting bodies produced in pure culture, when placed in a moist chamber. Binucleate oidia are borne in a drop of colorless liquid on the ends of the compact coremia. In addition, round, binucleate conidia are borne singly on tips of hyphal branches of the mycelium. On haploid mycelia, grown from single basidiospores, coremia, and conidia are formed also, but are smaller than the binucleate ones.

Progress in the Development of Eradicant Fungicides. G. W. KEITT.

Many plant diseases are combated chiefly by repeated applications of *protectant* fungicides. Usually enough infection occurs to occasion annually recurring primary inocula, often abundant and difficult to control. Studies here reported aim at developing *eradicant* fungicides adapted for direct attack upon pathogens at vulnerable stages. Strong eradicant fungicidal properties have been demonstrated for various mixtures of copper sulphate, lime, and certain arsenites (notably of calcium, copper, zinc, and iron) in water. Such mixtures release toxic materials over a long period. Early results from their use against *Venturia inaequalis* were reported to this society. Later results against several pathogens, mostly from small-scale experiments, follow: Spraying leaves in fall prevented or sharply limited ascospore production by *Ventura inaequalis, V. pyrina,* and *Coccomyces hiemalis.* Some preparations caused serious host injury: others did not. Late dormant spraying killed the perithecia of *Plowrightia morbosa* and *Mycosphaerella rubina,* and prevented or sharply limited production of conidia by *Sclerotinia fructicola.* High effectiveness against fruiting structures at or very near the surfaces of invaded tissues is demonstrated. Potential vulnerability of many pathogens to attack by surface applications of suitable eradicant fungicides is indicated. This report is made to invite efforts to improve and practically adapt eradicant fungicides.

Fungicidal Properties of Certain Copper-lime-arsenite Preparations. G. W. KEITT and D. H. PALMITER.

Toxicities of various preparations of copper sulphate, lime, and certain arsenites (especially of calcium) in water have been studied by a standardized cultural technique in which plant pathogens were incubated on plates of malt agar containing the toxic materials. Mono-, di-, and tricalcium arsenite, each used alone, equaled or exceeded copper sulphate in toxicity. The toxicity of copper sulphate-lime-calcium arsenite preparations equaled or exceeded the additive toxicities of the copper sulphate and calcium arsenite used in them. Such preparations showed high toxicity to all of 10 plant pathogens

tested. Copper-lime-calcium arsenite preparations showed powerful fungicidal action by materials that diffused through the agar. This was strongest in initially acid media, but also strong in media initially moderately alkaline. Each calcium arsenite, used alone, showed similar but less powerful fungicidal action. The copper-lime component, used alone, showed no perceptible fungicidal action by diffused compounds in alkaline media and very little in neutral or slightly acid media. The fungicidal properties of copper-lime-arsenite preparations vary greatly with modifications in relative amounts of the component materials and with the choice of arsenites, which have a wide range of solubility and other important properties.

Pathologic Significance of Seed-Coat Injury in Dent Corn. BENJAMIN KOEHLER.

A slight puncture in the seed coat at the crown caused a 12 to 16 per cent loss in yield of grain, while removal of the seed coat from the whole crown resulted in a loss of 18 to 23 per cent. A cut through the seed coat at the side of the kernel where it is underlain with horny endosperm caused no injury to stand or yield. Breaking away of the tip cap where the kernel is attached to the cap caused no apparent injury. Inoculation experiments in the field showed that, under wet soil conditions, *Aspergillus flavus* and *A. tamarii* caused marked injury to corn with seed coat injured at the crown, producing virescent seedlings, many of which died from lack of chlorophyll. Injury from *Gibberella saubinetii* was confined to dry soil conditions and was most pronounced in plots planted with seed injured at the crown. *Penicillium oxalicum*, also, was much more active on grains injured at the crown than on sound grains, under both wet and dry soil conditions. A seed treatment with ethyl mercury phosphate practically prevented all loss to stand or yield resulting from injured seed coats.

Heat Treatment for the Cure of Yellows and Rosette of Peach. L. O. KUNKEL.

Peach trees infected with either peach yellows or peach rosette virus may be cured by holding them at high temperatures. Cured trees remain healthy in appearance indefinitely, and graft inoculations from them to healthy trees do not cause disease. Experimental cures were obtained by incubating potted trees in a hot room at a temperature varying from 34.4° to 36.3° C. for 2 weeks or longer. Shorter periods of treatment were not uniformly effective. Exposures up to 3 weeks caused no serious injury to the trees. Since moderately high temperatures destroy these viruses, it is believed that the southern distribution of yellows and rosette may be determined by summer temperatures. Some difficulty was encountered in destroying the viruses in roots. The roots are embedded in earth that must be kept moist. They do not, on account of evaporation, reach the temperature of the tops. Retention of these viruses by rooots of treated trees suggests that the virus of phony disease may be confined to roots because of the high summer temperatures to which aboveground portions of trees are exposed in areas where this disease occurs.

A Phytophthora Wilt of Black-Locust Seedlings. E. B. LAMBERT and B. S. CRANDALL.

A wilt of black-locust seedlings caused considerable loss in 1933 and 1934 in a Virginia nursery. Consistent isolation, inoculation, and reisolation experiments proved that the disease is caused by a species of Phytophthora, which apparently can enter young and tender plant parts only. During the first 3 weeks after a seedling emerges the entire attacked plant wilts; but if it escapes until it is a month or more old, usually only the new growth wilts and the plant recovers. Drooping of the cotyledons, usually the first symptom, is rapidly followed by the collapse and shriveling of the entire seedling. The white swollen bases of the stems and the roots are the last to be affected, in com-

plete contrast with the damping-off symptoms that result from Rhizoctonia attack on the same host. In field experiments in 1934, the Phytophthora wilt was effectively controlled by scarifying the seed and spraying with Bordeaux mixture to protect the emerging seedlings and new growth. Four applications were made at the rate of 6 gallons per 400 square feet. The incidence of the disease was also greatly reduced by increasing the acidity of the upper 3 inches of soil from pH 6.2 to 4.6 with aluminum sulphate. (Bureau of Plant Industry and Emergency Conservation Work.)

Resistance to Fusarium Wilt in Muskmelon. J. G. LEACH and T. M. CURRENCE.

Muskmelon wilt, caused by a species of Fusarium and first observed in 1931, is slowly spreading through the melon-growing region near Minneapolis and St. Paul, Minnesota, and continues to be very destructive wherever it occurs. Numerous varieties of muskmelons have been tested for resistance on infested soil. The Honeydew and Persian melons were partially resistant, while other commonly grown varieties of muskmelon were very susceptible. One of about 30 plants selected in 1932 from a field of the Golden Osage type was apparently a chance hybrid between Golden Osage and Honeydew, and in 1933 yielded resistant plants of various types. Further selections were made and grown in 1934. Self-pollinated selections from these have been obtained for further study. The demonstration of resistance within the edible varieties of *Cucumis melo* lends encouragement to the selection of wilt-resistant types.

Uniform Rust Nurseries Indicate Decreasing Severity of Stem Rust. M. N. LEVINE and E. C. STAKMAN.

Observations on uniform rust nurseries, grown in various parts of the country and under diverse geographic and climatic conditions, show that there has been a gradual diminution in stem-rust losses during the past 15 years. While there was considerable variation from year to year, the severity of stem-rust infection was consistently decreased in both the wheat and oat nurseries, when comparisons were made by 5-year periods in the case of the former and by 4-year periods in the case of the latter. Reduction in severity of rust was further demonstrated by the reaction of individual varieties of wheat and oats in separate crop areas or in the entire nursery territory during the period under review. Several factors may be responsible for the reduction. During the past several years rainfall has averaged below normal. Nevertheless, some seasons were very favorable for rust development. Despite this, the losses were less than formerly. The eradication of about 20 million barberry bushes and the increasing use of resistant varieties of wheat and oats no doubt are very important factors in decreasing losses. (Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.)

Cripple and Dark Stripe of Pineapples in Hawaii. M. B. LINFORD.

A disease, apparently identical with "cripple" in Queensland, is discussed as to symptoms, developmental sequence, and occurrence in Hawaii. Cripple is a fruit deformity resulting from partial or complete failure to develop one or more fruitlets. Dark stripe, on either surface of foliage or modified leaves, is an isolated, narrow, brown to black band of tissue devoid of epidermis, showing periderm activity. Absence of epidermis results both from tearing apart of adherent organs and from failure of the meristem to differentiate an epidermis. Dark stripe on floral bracts and sepals is closely associated with cripple, that on involucral bracts less closely, and that on foliage remotely

if at all. Dark stripe and mild deformity have been recognized in young inflorescences long before blossoming, the deformity increasing during growth. Neither mechanical injury nor pathogenic microorganisms seem involved. Cripple has appeared no more frequently in vegetative progeny of affected than of unaffected plants. Both cripple and dark stripe appear to arise from faulty differentiation at the apical primordium, but the basic cause is unknown. Cripple occurs widely in Hawaii, ranging from a trace to several per cent. No influence of culture or weather has been recognized.

Relation of Carbohydrate-nitrogen Nutrition to Disposition of Apple to Infection by Erwinia amylovora. G. K. K. LINK.

Stayman trees furnished by G. T. Nightingale and of identical past experience were grown in sand culture in Chicago tanks. Complete or plus-nitrate solution was applied once to one lot; to the other, instead, a minus-nitrate solution. The former lot developed succulent shoots with large dark leaves; the latter, shorter, thinner, but woody shoots with smaller, fewer, and lighter colored leaves. Both sets of shoots were inoculated simultaneously with *Erwinia amylovora* by spraying or by hypodermic injection. At inoculation the past experience of plants of the two sets differed only in application of 2.08 gms. of nitrate per plant. The former, the relatively low carbohydrate-high-nitrogen plants, were positively disposed, susceptible; the latter, the relatively high carbohydrate-low-nitrogen plants, negatively disposed, resistant: a demonstration of the rôle of past experience as a hygio- and pathogenic factor, respectively, in etiologic complexes of health and disease in plants. Susceptibility of the plus-nitrate plants was maintained through the season by their development of new shoots below lesions. Consequently, a negative correlative factor, the influence of a lesion in inducing development of a new susceptible region, was the only experimental variable in the etiologic complexes of the second and subsequent sets of lesions.

Suitability of the Chicago Soil-nutrient Temperature Tank for Phytopathologic Studies. G. K. K. LINK.

The Chicago botanical laboratory has developed the Wisconsin soil-temperature tank into a soil-nutrient-temperature tank. Glazed earthenware percolator urns permit constant drip or periodic application of solutions to soil or sand cultures. Coupled with use of air-temperature control, the tank readily permits simultaneous control of soil nutrients, water, and other hygiegenic and pathogenic soil ingredients; of soil pH and temperature; of air temperature; and coupled with use of shades and lamps, of carbohydrate nutrition. The containers avoid direct pathogenic effects of improper soil aeration, metal ions, and restricted root development and their indirect pathogenic effects in disposing plants to pathogenic influences. G. T. Nightingale, of the New Jersey Experiment Station, used the tanks at Chicago, demonstrating their suitability for analysis of the relation of ecic (environmental) factors and of the processes, structures, and behavior of apple trees. The author used them for study of the relation of carbohydrate-nitrogen nutrition of apples to disposition to infection by *Erwinia amylovora*, demonstrating their suitability for analysis of the etiologic complexes of infections of aerial parts of plants. Goldberg and the author, using sand cultures and varieties of cabbages resistant and susceptible to infection by *Fusarium conglutinans*, demonstrated suitability of the tanks for study of infection by soil-borne organisms.

Variability of Monosporic Cultures of Coccomyces hiemalis. ROBERT O. MAGIE.

The variability of 20 isolates of *Coccomyces hiemalis* from 6 States was studied. Length of conidia varied much within and between isolates. In culture the isolates

showed considerable variation in colony color and contour, growth rate, acid production, and spore production, both conidial and microconidial. No significant variation was found between the isolates in their utilization of various inorganic and organic sources of nitrogen and of several sources of carbon. Slight variation between isolates was demonstrated in the relation of temperature and hydrogen-ion concentration to growth and conidial germination. Conidia germinated over a range of pH from 2.9 to 8.5. Growth and production of conidia on agar occurred at temperatures from 4°–28° C.; none at 32°. The optimum temperature for growth lay between 20° and 24° C., and for conidial production at 12° or 16° C. Over a dozen isolates tested for pathogenic variability on several Prunus species were relatively uniform in their reactions, abundant infection occurring only on sweet and sour cherry. The isolates from any one locality varied as much as those from widely different localities. The existence of well-defined physiological forms of the fungus was not indicated.

Microorganisms in the Atmosphere of Arctic Regions. F. C. MEIER.

The Lindbergh Atlantic Survey Flight of 1933 presented an opportunity to obtain significant data concerning long-distance aerial movement of spores of fungi and pollen. Cooperating with the writer in studies on the relation of air-borne spores of fungi to the epidemiology of plant diseases, Colonel Charles A. Lindbergh, in the summer of 1933, made 26 collections of material present in the atmosphere at various altitudes over stretches of water and ice along his course of flight between Maine and Denmark. A device designed especially for exposing petrolatum-coated microscope slides from the low-winged monoplane *Tingmissartoq* while in flight, with slide containers so constructed as to prevent contamination before and after exposure, was used. Detailed notes and maps made at time of exposure provide a basis for interpretation of results as later revealed by direct examination of the slides under the microscope. The slides show certain spore types to be abundant in the air currents over Maine and Labrador and present in diminishing numbers as collections progressed to leeward over Davis Strait, the great ice cap of Greenland, and Denmark Strait.

Evidence of the Overwintering of Plasmopara australis in Fruits of Sicyos angulatus. I. E. MELHUS and G. C. KENT.

Plasmopara australis occurs annually on *Sicyos angulatus* growing on islands in the Mississippi River. Its abundance on the cotyledons of seedlings in late May and early June suggested the possibility of seed transmission. The mortality in the seedling stage is very high with abundant conidial sporulation. In the fall of 1933 the green fruit was found infected. Yellow areas and characteristic conidiophores were conspicuous symptoms. Material killed and fixed showed abundant mycelium in the ovary wall with conidiophores breaking through the outer and inner ovary wall. The conidiophores breaking through the inner ovary wall produced spores abundantly within the cavity between the ovary wall and the single mature seed. Spores were often found free in this cavity. There was no indication of oospores or of mycelium in the seed. The ovary wall encloses the seed until germination, which suggests a method of seedling infection.

A Practical Method of Organization for Long-time Phytopathological Field Experiments. I. E. MELHUS and C. S. REDDY.

An Albino Strain of Barley Smut. M. B. MOORE and C. C. ALLISON.

In the summer of 1934 a single head of barley infected with what appears to be an albino strain of *Ustilago hordei* was found growing in one of the field plots at University

Farm, St. Paul, Minnesota. The head, almost white, was intermediate between the loose-smut and the covered-smut types. The hyaline spores were smooth, somewhat smaller than those of normal *U. hordei,* and produced promycelia and sporidia on germination. Sporidial fusions between monosporidial lines indicate 2 sex groups within the albino strain that are compatible with those of normal *U. hordei* and *U. medians.*

The Distribution of Intermediate Types of Barley Smuts. M. B. MOORE and C. C. ALLISON.

To determine the distribution of barley smuts, about 700 samples of barley seed were obtained from Minnesota and about 125 from other States. Seed from these samples was sown in 1934 and the smutted heads, which appeared in about half of the plots, were examined for head type and color of spore mass. The spore-wall character and type of germination of the chlamydospores also were determined. Smutted heads collected in barley fields were similarly examined. Results show that, in addition to *Ustilago hordei* and *U. nuda, U. medians* is widely distributed from North Carolina to Colorado and from Missouri to Minnesota. More than one kind of smut often occurred in the same sample, occasionally two in the same head. Chlamydospores in one "loose smut" head were smooth and produced sporidia on germination. *U. nuda* and *U. medians* could not be differentiated by head type or color of spore mass. Heads determined as being smutted by *U. nuda* fell into at least two color groups.

Effect of Crown Rust on the Cold Resistance of Oats. H. C. MURPHY.

Winter-oat varieties and selections infected with crown rust were more susceptible to injury from artificially produced low temperatures than similarly hardened rust-free plants. Although the varieties and selections differed greatly in relative resistance to sub-freezing temperatures, it was always possible to select an exposure that was fatal to all infected plants of a particular variety or selection and that would allow a complete survival of rust-free ones. Plants with 10 per cent infection were easily differentiated. The heavier the infection, the greater was the susceptibility to injury. Tennessee numbers 090, 1918, 1962, 1922, and 1945 (C. I. Nos. 3175, 3172, 3174, 3171, and 3168, respectively) developed from Fulghum (winter type, C. I. 2499) and supplied by N. I. Hancock, were the most resistant of 30 selections studied, to low temperatures and were more resistant than any of 30 standard winter varieties. Hairy Culberson (C. I. 2505) and Culberson (C. I. 273), Coker 32–1 (C. I. 3026), Winter Turf selections (C. I. 435–4 and 409–5), Fulghum (winter types, C. I. Nos. 2499 and 2500), and Culred (C. I. 518–189) were the most resistant standard varieties. Some varieties hardened more quickly than others. Certain volunteer hybrid selections withstood −16° F. under field conditions at Ames, Iowa, and survived after being transplanted to the greenhouse on January 24, 1934.

Effect of Crown Rust on Yield, Water Economy, and Composition of Oats. H. C. MURPHY.

Crown-rust infection of oats, initiated in the seedling and boot stages, entirely inhibited production of grain of the variety Markton (susceptible) and reduced the yield of Victoria (resistant) 47.5 and 30.8 per cent, respectively, while anthesis-stage infection reduced the yield of Markton 45.3 and of Victoria 18.3 per cent. Late dough-stage infection produced no significant effect. Root yield was reduced most and straw yield least. Susceptible plants infected in the seedling stage used 290.8 per cent more water per unit of dry weight than rust-free plants; resistant ones used 39.9 per cent more. Infection reduced the ratio of roots to tops. Effect on yield, water requirement,

and root-top ratio was in proportion to duration and severity of infection. Except for slight increase in crude protein and decrease in nitrogen-free extract for kernels, the composition of kernels and hulls was unchanged. Green, infected plants of Markton, Iogold (susceptible), Victoria, and Bond (nearly immune) showed an increase in percentage content of insoluble solids, soluble and insoluble ash and nitrogen, and acid-hydrolyzable substances, and a decrease in soluble solids, sucrose, glucose, levulose, and dextrin. The sugars were influenced most, levulose showing a decrease of 94.7 per cent in the infected Markton plants. The changes were in proportion to percentage and type of infection.

Three Forms of the Fusarium Wilt of Celery. RAY NELSON and L. C. COCHRAN.

Two forms of Fusarium wilt or yellows of celery occur generally in the celery districts of Michigan and also in a number of other States. An apparent third form, the distribution of which has not yet been determined, has recently been observed in California. These forms of Fusarium wilt have some characteristics in common, but are readily separated by the type and sequence of the initial symptoms in the leaflets. Of the two that are widely distributed the initial symptoms in the leaflets of form I are: (a) a progressive yellowing of the lamina, with the veins remaining unaffected much longer than the intervenal areas; (b) an absence of curl. In form 2 the initial symptoms include: (a) downward curling of the leaflets; (b) blanching of color from the veins and a narrow band of immediately adjacent tissue. The intervenal areas blanch much more slowly and the heart leaflets are seldom decolorized. In form 3 the leaflets usually are neither curled nor decolorized but the plants are dwarfed and exhibit other symptoms that show its relation to the other two forms of the disease.

Taxonomy of the Fusaria that Cause Celery Wilt (yellows). RAY NELSON and L. C. COCHRAN.

The pathogenic Fusaria associated with the wilt or yellows disease of celery fall into at least 2 groups. Two of these have been studied extensively and are morphologically indistinguishable from each other, nor are they clearly distinguishable on morphological grounds alone from other parasitic members of the subsection Orthocera. A third group is being studied, which apparently differs from the other two in certain morphological characters. The parasitic members of the subsection Orthocera differ morphologically from one another only in certain minor details. Apparently no great importance can be attached to the slight and variable differences in spore sizes, and specific separation on this basis appears unwarranted. The logical taxonomic procedure is to recognize only one species, *Fusarium lini*, and the other members as forms of this first named species. This treatment however, would contribute nothing to a better understanding of the relationships, nor would it provide a satisfactory classification for plant pathologists. Where clearly recognizable morphological differences are lacking, primary consideration should be given to host relationships in classification of the parasitic Fusaria. On this basis the 2 clearly recognizable groups of celery Fusaria are given, respectively, a specific and a varietal name, *Fusarium apii* and *Fusarium apii* var. *pallidum*. A third group requires further study.

A Study of Electric Soil Sterilization. A. G. NEWHALL.

Two general types of box sterilizers have been compared: the indirect, heating element, or New York type, and the direct, resistance, or Ohio type. Various kinds of soil, from pure quartz sand to muck, have been treated in over 100 test runs. Energy consumed was metered and reduced to kilowatt hours per cubic foot of soil per 50-degree

rise in temperature (21° to 71° C.), which amounted to less than 1.3 kw. hour. The optimum initial soil moisture, for most soils, was above 2/3 of its water-holding capacity d.w.b. The time required to raise the soil mass to 70° C. varied between 30 minutes and 6 hours, depending upon the sterilizer, soil type, its moisture and soluble salt content, and initial temperature. The following organisms have been placed in soils and killed, in varying lengths of time, by raising the soil temperature to 71° C. or less; *Heterodera marioni, Bacillus carotovorus, Rhizoctonia solani,* from several hosts, *Sclerotinia sclerotiorum, Phytophthora cactorum, Pythium ultimum,* and *Fusarium* sp. Ordinary soil-heating cable did not prove adequate for sterilization purposes, but pipe-type space heaters have been used in a limited way in bench soil.

A Study of the Carotenoid Pigments of Urediospores of Wheat Stem Rust and Four of its Color Variants. MARGARET NEWTON, H. JOHANNSON and T. JOHNSON.

Urediospores of stem rust and of four color variants of it (greyish brown, orange, white, and antique brown) were developed on wheat plants grown under normal and subnormal light intensities. Spectrophotometric determinations of the pigment concentrations of the naphtha extracts of spores of the above color types showed for each type a higher pigment content in spores produced under normal than under subnormal light. The concentration of pigment varied with the color type. Arranged in descending order of pigment concentration the types stood as follows: orange, normal, antique brown, white and greyish brown. Spectral distribution curves indicated that the pigments were carotenoid in nature, and in most instances apparently consisted chiefly of carotene.

A Cytological Study of the Resistance of Apple Varieties to Gymnosporangium juniperi virginianae. C. J. NUSBAUM.

Infections by *Gymnosporangium juniperi virginianae* in the leaves of 4 apple varieties, each differing from the other in its reaction to the rust fungus, were studied cytologically. The process of penetration by sporidial germ tubes was essentially the same on each variety. The fungus began its parasitic activities intracellularly by forming a conspicuous primary hypha in an epidermal cell. Further development of the mycelium was regularly intercellular, with the formation of characteristic haustoria. In Wealthy leaves of susceptible age, the parasite developed vigorously in congenial relationship with the host. As long as the invaded cells remained alive, they apparently furnished a favorable substrate. The resistance shown by the other varieties used seemed to be caused by a distinct antagonism of the host-cell protoplasts to the invading parasite. This reaction was mild in Yellow Transparent, pronounced in Fameuse, and very severe in Baldwin. Wealthy leaves became resistant as they matured. The fungus penetrated and made considerable vegetative growth in leaves that had just attained full size, but failed to initiate pycnia, in spite of an apparently congenial relation between the host and parasite. The sporidial germ tubes were unable to penetrate the epidermal cells of leaves several weeks older.

Dissociation of Fusarium niveum in Soil. C. R. ORTON.

During the months of March and August, 1933, 48 2-liter Ehrlenmeyer flasks were half filled with greenhouse soil of a sandy-loam texture. These were steam sterilized and 42 of them infested with 9 strains of *Fusarium niveum* from different sources. Six flasks were held as checks. The prepared flasks were distributed as follows: 16 in laboratory, 16 in greenhouse and 16 in a 6-inch soil trench outside. At approximately monthly intervals, samples were carefully removed from each flask and plated on agar.

Each colony thus obtained was studied in comparison with its respective "parental" strain. Three groupings were made: (1) colonies like parental strain; (2) colonies unlike parental strain, hereafter referred to as "colony dissociants"; and (3) colonies showing sectorial dissociation. Analysis has been made of 8,220 colonies isolated from the 48 soil flasks. Colony dissociants appeared 25 times and "sectorial dissociants" 16 times from 7 strains. Two strains have produced no dissociants. All of the remaining colonies were like the parental strains. Of the 25 colony dissociants 12 were distinctive—the other 13 representing replications. Of the 16 sectorial dissociants, 13 were distinctive. A number of the colony dissociants were apparently like sectorial dissociants obtained from the soil and/or stock cultures carried on plates in the laboratory.

Incubation of the Virus of Pea Mosaic in the Aphid, Macrosiphum gei. H. T. OSBORN.

The virus of pea mosaic, which has already been shown to require about 12 hours of incubation in the aphid *Macrosiphum pisi*, before it can be transmitted by this carrier, undergoes a similar period of incubation in the vector *M. gei.* An interval of not less than 12 nor more than 18 hours was found necessary for the proper incubation of the virus in this insect. The pea-mosaic virus is thus proved to enter into a specific relationship with two different aphids. A second virus affecting peas and other legumes, designated as pea-mosaic virus No. 2, is readily transmitted mechanically and is spread by both *M. pisi* and *M. gei.* This virus is transmissible by *M. pisi* within 30 minutes after being picked up, and is usually not retained for more than an hour. Its transmission by this vector seems to involve only a mechanical transfer.

Physiologic Studies of Several Pathogenic Bacteria that Induce Cell Stimulation in Plants. J. A. PINCKARD.

Atypical and pathological stimulation of plant cells has been investigated through certain bacteriological and physico-chemical studies of single-cell cultures of bacteria causing crown gall, olive tubercle, oleander tubercle, bacterial-pocket disease of sugar beet, and of an unnamed bacterium causing overgrowths on canes of black raspberry plants. The pathogenicity of the cultures varied on different host plants. The results of common bacteriological tests were usually differential. The optimum temperature for growth of all of the cultures on agar was near 28° C. They varied in amounts of growth with a number of sources of carbon and nitrogen. Alkaline reactions consistently occurred where starch and salts of organic acids were the sources of carbon. Marked differences in acid reaction developed where various other sources of carbon were present. Similar differences were noted in media containing several nitrogen sources. With oxamide, l-tyrosine and l-cystine an acid reaction was consistently induced by all of the cultures. Potentiometric oxidation-reduction studies in conjunction with H-ion determinations with glass electrodes showed that no limiting factors of pH were present in any of the media used and that strongly reduced potentials developed in undisturbed liquid cultures.

An Improved Method for Inducing Spore Fructification in Certain Species of Macrosporium. P. P. PIRONE.

In the course of studies on the leaf blight of carrots, caused by *Macrosporium carotae*, an improved method for inducing spore formation was developed. Air-drying of cultures for 24 to 48 hours at 21° C., with a relative humidity of 30 to 40 per cent, resulted in the production of large numbers of conidia. The age of the cultures used varied from 5 days to 4 weeks. They were grown on carrot, corn-meal, and potato-dextrose agars. The spores appeared in the air-dried dishes 48 hours after the Petri-dish covers were replaced. Undried cultures from the same source and of the same

age produced few to no spores. The present method produced a greater number of spores than did the wounding and shredding of young cultures with a hot scalpel as suggested by other workers. Danger of contamination by exposure was eliminated largely by use of a cellophane tent or covering placed a few inches above the exposed plates.

Bacterial Wilt of Corn and Its Insect Vectors. F. W. POOS and CHARLOTTE ELLIOTT.

During April, 1934, *Aplanobacter stewarti* was isolated from overwintered adults of the flea beetle, *Chaetocnema pulicaria*. Isolations have been made from about 40 species of insects, and *A. stewarti* has been isolated only from *C. pulicaria*. As many as 75 out of 100 *C. pulicaria* were found to contain *A. stewarti* when collections were taken from different host plants and localities under varying conditions in the field. Previously published reports of the transmission of *A. stewarti* from infected to healthy corn plants by means of the adult beetles of *C. pulicaria*, *C. denticulata*, and *Diabrotica duodecimpunctata* have been verified. Attempts to obtain infected plants in field cages by planting the seed in soil (both sterilized and not) inoculated with *A. stewarti* at planting time yielded only negative results. Bacterial-wilt lesions have been found on teosinte, *Euchlaena mexicana*. Bacteria isolated from these lesions have produced the disease on corn. Adult beetles of *C. pulicaria* also transmitted *A. stewarti* from infected corn plants to healthy teosinte, and pure cultures of *A. stewarti* were isolated from these infected teosinte plants.

Some Environmental Factors Influencing the Development of Apple Hairy-root and of Phytomonas rhizogenes. A. J. RIKER, D. H. PALMITER and E. M. HILDEBRAND.

The influence of temperature on the development of hairy root was studied during 4 spring seasons on 1-year-old Snow apple trees grown in Wisconsin temperature tanks. The trees were inoculated with *Phytomonas rhizogenes*, through wounds below the soil line. They were grown approximately 10 weeks in soil maintained at 75 per cent of its moisture-holding capacity and at 12°, 16°, 20°, 24°, 28°, and 32° C., respectively. The longest shoot growth appeared between 16° and 24° C. The largest hairy-root growth and the highest percentage of infection occurred at approximately 24° and 28° C. In the greenhouse kept near 24° C. inoculated trees were grown in soil at 60, 75, and 90 per cent of its moisture-holding capacity. Hairy-root development was slightly greater at 90 per cent, but the percentage of infection was slightly higher at 75 per cent. Colonies of the causal bacteria was grown on plates of yeast-infusion mannitol agar and of carrot-extract agar incubated, respectively, at temperatures between 4° and 36° C., inclusive. Widest colony growth was found at 28° C. In a yeast-infusion mannitol liquid medium the most acid and the most dense turbidity were produced also at 28° C.

Copper Phosphate Mixture: A Promising Fungicide. J. W. ROBERTS, L. PIERCE, M. A. SMITH, J. C. DUNEGAN, E. L. GREEN, and M. C. GOLDSWORTHY.

Field trials on apple, pear, and cherry during the 1933 and 1934 seasons at Beltsville, Md., Vincennes, Ind., Springfield, Mo., and Fayetteville, Ark., have indicated that the mixture composed of 2 pounds of copper phosphate (tri-cupric phosphate), 2 pounds of bentonite, and 4 pounds of hydrated lime in 50 gallons of water is a promising fungicide. The mixture has controlled mild to medium cases of apple scab, cherry leaf spot, and Fabraea leaf blight of pears, and has not caused injury to any of the leaves of these host plants. Slight russeting of fruit occurs with certain varieties. Slight stimulation was noted with all 3 hosts. The mixture has not been subjected to severe tests as a fungicide, and further trials are needed to establish its efficiency under the most favor-

able conditions of infection. Insecticides, spreaders, and stickers, of common use, may readily be incorporated in the mixture.

The Effect of Proteoclastic Enzymes on Purified Preparations of Tobacco Mosaic Virus. A. F. ROSS.

The virus was inactivated by trypsin. Heating the digests to 70° C. for 20 minutes resulted in a recovery of most of the virus. When enterokinase, calcium sulphate, calcium chloride, ammonium sulphate, magnesium sulphate, and barium chloride were used as activators for trypsin, permanent inactivation of the virus did not occur. It was impossible to adjust the H-ion concentration of the trypsin digests, within the limits that the virus remains active, so that permanent inactivation would occur. A combination of trypsin and pepsin was less effective in inactivation of the virus than trypsin alone. Papain inactivated the virus but it is not known whether or not this was due to adsorption or cleavage. Mixed cultures of bacteria caused a slow inactivation of the virus. Cultures of *B. proteus* and *B. aerogenes* were no more effective in inactivation than a mixed culture. Trypsin apparently attacked no protein that may be associated with the virus. Mixed cultures of microorganisms also were ineffective in digesting away any material associated with the virus.

Chemical Splitting of the Tomato "Combination-Streak" Virus Complex. MICHAEL SHAPOVALOV.

Expressed tomato or tobacco juice from plants infected with the "combination-streak" virus was mixed with equal amounts of different concentrations of a number of viricidal substances. Of the two components of this virus complex, green tobacco mosaic, known as Johnson's virus No. 1 (also sometimes referred to as virus A) showed, as a rule, higher resistance to these chemicals than latent potato virus (sometimes referred to as virus B). The usual method of separating this latter virus from the complex consists in employing such differential hosts as are subject to systemic infection with virus B alone. In a few chemical tests, however, certain sulphates, namely, $CoSO_4$, $NiSO_4$ and $ZnSO_4$, at concentrations incapable of producing lytic effect on the whole complex, appeared to be partically toxic to both component viruses. As a result, virus B occasionally survived, while virus A was inactivated, and *vice versa*. The limited number of trials thus far conducted show no regularity in this effect, and the outcome of these trials can not be predicted.

Influence of Nutrition on Host Susceptibility to Yellow Tobacco Mosaic. E. L. SPENCER.

Experiments have shown that susceptibility of seedlings of *Nicotiana tabacum* var. Turkish to yellow tobacco mosaic (Johnson's tobacco virus 6) can be influenced markedly by the addition of various amounts of nitrogen, phosphorus, and potassium. Plants were grown in 4-inch pots in a soil of low fertility and fed twice each week with their respective nutrient solutions. The pin-puncture method of inoculation was used. Susceptibility was measured by the number of lesions for each 100 pin punctures. Growth was measured by recording the green weights of representative plants at time of inoculation. Results showed that host susceptibility to virus infection in tobacco is definitely correlated with host nutrition. In the nitrogen study, plants that grew most rapidly were considerably less susceptible than those whose growth was retarded by excess nitrogen. In the phosphorus study, susceptibility increased when growth increased and decreased when growth fell off, due to excess phosphorus. In the potassium study, susceptibility was greatest in plants receiving small amounts of potassium but decreased sharply with

the addition of larger amounts; growth did not fall off appreciably until very large quantities of potassium were added.

Population Trends of Physiologic Forms of Puccinia graminis tritici, 1930 to 1934. E. C. STAKMAN, LEE HINES, ROBERT C. CASSELL, and M. N. LEVINE.

There have been striking changes in the relative prevalence of physiologic forms of *Puccinia graminis tritici*, based on determination of several hundred collections of rust in Mexico and the United States annually from 1930 to 1934, inclusive. In 1930 form 56 constituted only 0.2 per cent of all isolates, but its prevalence increased gradually until 1934, when it suddenly became the most prevalent of the 22 forms identified, constituting more than 30 per cent of the isolates. Similarly, the prevalence of form 34 increased from 0.7 per cent in 1930 to about 20 per cent in 1934. Form 139, first identified from an artificial cross, also seems to be increasing, but not so rapidly. On the other hand, the prevalence of form 38 decreased from an average of 34 per cent for the first 4 years to less than 4 per cent in 1934. There has been considerable fluctuation in the relative prevalence of other forms. It seems significant that all forms were identified from 29 aecial collections in 1934, while 21 forms were identified from about 700 uredial isolates. The ratios of forms to isolates therefore are about 1: 3 and 1: 33, respectively. (Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Experiments on Physiologic Specialization and Nature of Variation in Ustilago zeae. E. C. STAKMAN, L. J. TYLER, G. E. HAFSTAD and E. G. SHARVELLE.

Six varieties of corn were inoculated hypodermically with 10 collections of *Ustilago zeae* three successive years. The collections differed consistently in pathogenicity. Because of the nature of resistance, however, physiologic specialization now appears relatively unimportant in breeding resistant lines of corn. Frequency of sectoring was studied in monosporidial lines derived from successively budded sporidia. In general, sectoring did not decrease appreciably, even in a line derived from the 13th successive sporidium. Apparently, sectoring was not due to delayed segregation. That tendency to sector is genotypically determined is shown by the following: On a promycelium segregation was of the 1, 2; 3, 4 type, culturally and sexually. In 89 colonies of 49 monosporidial lines from sporidia 3 and 4 there were 360 sectors; in about the same number of colonies of lines from sporidia 1 and 2 there were none. There were 8 types of segregation in 11 chlamydospores from a certain cross, including 3 that apparently remained diploid. Some monosporidial lines from the cross appear stable and are being combined to ascertain the importance of hybridity in sectorial variation.

Studies on the Soft-rot and Colon-typhoid-dysentery Groups of Bacteria. 2. Physiology. A. R. STANLEY.

A series of physiologic tests extending over a period of about two years were carried with 120 organisms of these groups. Cleavage of sugars, reduction of nitrates, production of indole, growth in Koser's citrate solution, methyl red test, and Voges-Proskauer test were tried. The indole test was the most consistent. Only one culture, after producing indole for some time, ceased to give a positive test in subsequent trials. It was found that 38 of these changed with respect to acid and gas production in one or more of the sugars. In later tests some of these reverted to their original reaction; while some have remained constant, a few have undergone further changes. Thus a

single organism could be classified at different times in two or even three different genera, according to Bergey's Manual. Our studies with these closely related bacteria indicate the inadequacy of the present classification (Bergey) as a basis for identification. Neither pathogenicity nor physiologic reactions can be fully relied upon for specific identification with such variable forms.

A Method of Determining the Rate of Decay in Wood. F. H. STEINMETZ and M. T. HILBORN.

The comparative loss in calorific value is suggested as a method of determining the rate of decay in wood. Since calorific value depends upon the chemical nature of the cell walls and cell contents, as well as upon the total amount of substances present, the method appears to be applicable. The actual combustion sample used was approximately 1 gram of pulverized oven dry wood or wood residue. Data are presented to illustrate the amount which fuel wood depreciated in calorific value over a 3-year period. The calorific values are based upon representative samples taken from pieces of split cord wood that had been stored in an open field. Three species of fuel wood were used in the experiment. The rate of loss is illustrated by the values obtained for red maple. By using $8.00 as the value of sound dry wood per cord cut in January and February the values for the 4 successive autumns were found to be $5.50, $3.40, $2.90, and $1.73 per cord. When expressed in percentages based upon millions of B. T. U. per cord, the efficiency of decayed wood was 70, 60, 59, and 38 for the 4 successive autumns following the time of cutting.

Further Evidence of the Seed-borne Nature of Peronospora destructor. W. W. STUART and A. G. NEWHALL.

Indirect evidence has accumulated indicating that various members of the Peronosporales are seed-borne. But, so far, only in the case of blue mold of tobacco and downy mildew of beet have infected seedlings been obtained from seed suspected of harboring the fungus. After repeated failures in the greenhouse to obtain diseased seedlings from onion seed suspected of harboring *Peronospora destructor* the authors were successful in one outdoor planting under conditions that were thought to point strongly to the seed-borne nature of this disease. Seed heads of Yellow Globe stock were inoculated early in the morning in the fall, while still in blossom, with a conidial suspension of *Peronospora destructor.* Natural infection also took place freely from a near-by onion field. These heads were hand-thrashed, fan-cleaned, and sown the following spring in 2 isolated virgin muck fields. On one of these plots, surrounded on 3 sides by dense woodland and 1200 feet distant from the nearest field of onions, mildew developed at about the same time that it was observed in other commercial plantings in the county. Fresh onion seed has been found to withstand treatment for 20 minutes in hot water at 55° to 57° C., but the effect on Peronospora is not yet known.

Laboratory and Field Studies on Sulphur as a Fungicide. J. J. TAUBENHAUS and PHARES DECKER.

A research program on uses for sulphur and sulphur combined with other products as fungicides for control of plant diseases has yielded some interesting results. Laboratory studies indicate that sulphurs of a 300 mesh are highly toxic to spore germination of pathogenic fungi. The toxicity is considerably increased by adding to the sulphur small percentages of other standard fungicides or insecticides. Sulphur may thus be made a valuable carrier to take the place of inert material as at present used in some fungicide or insecticide dusts. Sulphur applied broadcast to some of the calcareous po-

tato soils of Texas failed to give appreciable control of potato scab, while fair control was obtained with sulphur applied in the furrows with the seed pieces. Sulphur or sulphur in combination with Cuprocide or copper sulphate applied to sorghum seed heavily inoculated with smut spores of *Sphacelotheca sorghi* gave good control. Sulphur as a dust on the foliage of a number of plants controlled powdery mildew, and mosaic of the Lima bean and snap bean, presumably because of controlling red spider and leaf hoppers on the treated plants. Two to four per cent of sulphur worked into the ground, or sulphur slabs of pure sulphur, made perfect barriers to prevent the encroachment of Phymatotrichum root rot from an infested to a noninfested area. A large number of plants were dusted periodically with sulphur without injury to the foliage, while the leaves of most varieties of cantaloupes have been burned severely by sulphur dusts.

Further Studies on the Longevity of Sclerotia of Phymatotrichum omnivorum. J. J. TAUBENHAUS and W. N. EZEKIEL.

Tests were begun in 1929 to determine how long sclerotia of *Phymatotrichum omnivorum,* removed from the field and placed in small vials of moist soil in the laboratory, would remain viable and virulent. Each year some of the sclerotia have been removed from the vials and tested for germination. In 1934 18 per cent. of the sclerotia germinated after storage for 5 years at room temperature.

Particle Size of Three Strains of Tobacco-Mosaic Virus. H. H. THORNBERRY.

Particle size of tobacco-mosaic, aucuba-mosaic, and masked-tobacco-mosaic viruses was determined by ultrafiltration through Elford's type of collodion membranes. These viruses are considered to be related strains, although they cause diseases differing in severity of symptoms. In finding the end point of ultrafiltration (the smallest pores that allow virus to pass), the amount of virus in the filtrates was measured by the local-lesion method on Scotia beans. Under favorable conditions for filtration (0.1 M Sörensen's phosphate buffer at pH 8.5 and 10 per cent broth), membranes with pores .0905 μ in diameter allowed a small amount of each virus to pass. Membranes with pores .0329 μ in diameter retained the viruses. According to Elford's assumption, virus particles greater than ⅓ the pore diameter do not pass the membranes. On this basis, the virus particles of each strain were found to have a diameter not greater than .03 μ or 30 mμ.

Fasciation of Sweet Peas: A Bacterial Disease. P. E. TILFORD.

A bacterial organism, distinctly not *Bacterium tumefaciens,* has been isolated from numerous greenhouse-grown sweet-pea plants affected with the disease that has been called fasciation. Inoculation experiments have proved the pathogenicity of the organism to several varieties of sweet peas and to garden peas. The organism is seed-borne. Affected plants have many short, fleshy, thick, and aborted stems with misshapen leaves growing from the main stem at, or below, the soil line, or from the hypocotyl region. The main stem appears to grow in a normal fashion, except that it usually is somewhat dwarfed. No lesions of any kind have been noticed in connection with the disease on young plants. The identity of the organism is being worked out.

Severe Type of Mosaic on a Sugar-cane Variety. E. C. TIMS.

Of the 2 mosaic types occurring on a sugar-cane variety (C. P. 28–70) in Louisiana, one of them causes much more severe injury than is generally attributed to the mosaic disease. This type or strain of mosaic produces a very conspicuous, yellow mottling of the cane leaves, which are also greatly reduced in size. The infected stalks also are severely stunted. Yield tests made over a period of 2 years showed that tonnage was

reduced about *32 per cent* and the sucrose content of the juice was reduced *20–30 per cent* by this severe type of mosaic. The loss in tonnage is due to reduction in size rather than number of stalks. The yellow type of virus may be transmitted to other cane varieties on which the symptoms are similar to those of the ordinary green type of mosaic. The green or mild type of mosaic on the same variety (C. P. 28–70) apparently causes little or no reduction in growth and only a slight reduction in sucrose content.

Do Tobacco Plants Recover and Develop an Immunity from Ring Spot? W. D. VALLEAU.

The following hypothesis is suggested to explain so-called recovery and immunity from ring spot: Tobacco plants inoculated with the ring-spot virus develop ring patterns in inoculated leaves and other leaves until invasion of the growing point is complete. The Liesegang effect appears to result from the invasion of a mass of healthy tissue. Leaves developed subsequent to complete invasion are without patterns, but are parasitized and are highly viruliferous, as in any virus disease in which patterns are undeveloped. The upper leaves of a "recovered" ring-spot plant are darker green; growth is retarded; and, at low temperature, young leaves develop leaf-edge chlorosis and necrosis. Finally, abortive pollen is produced. Yellow ring spot is identical with ordinary ring spot, except that patternless leaves bleach to a yellowish green, proving that recovery has not taken place. Inoculation of patternless leaves is without effect, as the cells are already parasitized to the limit. An acquired immunity is not involved. For the same reason, tobacco plants inoculated with a mild strain of tobacco mosaic are protected against a more injurious strain of the same virus.

The Resistance of Ambalema Tobacco to Different Viruses. W. D. VALLEAU.

Nicotiana tabacum var. Ambalema, found to be highly resistant to ordinary tobacco mosaic by Nolla, is susceptible to cucumber mosaic and green and yellow ring spot and slightly resistant to the etch viruses (smallest growing-point leaves free from virus). Ambalema plants were inoculated with 27 strains of tobacco mosaic, each differing in symptom expression. The majority caused only faint vein clearing, occasional faint patterns and yellow blotches, but the plants, on the whole, appeared healthy. Four strains caused chlorotic (in one virus necrotic) Liesegang patterns, often covering the leaf. The ring-spot patterns evidently resulted from slow penetration of healthy leaves, as in nearly every case the growing-point leaves were virus-free. Ambalema appears to separate strains from a given supposedly stable tobacco-mosaic virus, the process appearing not unlike that involved in the production of so-called attenuated strains by heat. An Ambalema plant may be shown to have been inoculated successfully several consecutive times if young leaves are used, and a strain of tobacco mosaic employed that causes local chlorotic patterns. A strain of tobacco mosaic (white) collected in Kentucky and the English aucuba mosaic each produce necrotic spots and concentric necrotic ring patterns on inoculated leaves, the virus appearing to be localized.

Technique Advantageous for the Isolation of Ceratostomella ulmi from Bark Beetles. J. M. WALTER.

Attempts to isolate the Graphium stage of *Ceratostomella ulmi*, the Dutch elm-disease fungus, from bark beetles by the agar-plate method have been highly unsuccessful because the agar is quickly overrun by fast-growing fungi, such as species of Mucor, Aspergillus, Monilia, and Fusarium. These apparently occur in the alimentary tracts, because washing the beetles for 5 minutes in 1: 1000 $HgCl_2$ did not eliminate them; nor did such modifications of common media as adjusting pH, reducing sugar content, and changing nitrogen base. Recently it has been found that the coremia of *C. ulmi* develop well and

that other fungi are checked if the beetles are crushed on sterilized chips cut from seasoned, debarked, elm branches about $\frac{3}{8}$ inch in diameter. Wood seasoned for 2 or 3 months is less favorable for growth of the unwanted fungi than is freshly cut wood. The chips should preferably be autoclaved dry on 2 or 3 sheets of filter paper in Petri dishes. Sufficient sterile water to moisten the chips and filter paper thoroughly is added a few minutes before the plates are used. This method also has proved advantageous in the isolation of *C. ulmi* from dead wood and bark. Several other species of Graphium, not easily isolated by the agar-plate method, have been also secured. (Emergency Conservation Work in cooperation with U. S. Department of Agriculture.)

An Aerial Rhizoctonia on Beans. G. F. WEBER.

A bean disease, new in the United States, was observed in epidemic form during the past 2 years in Alachua County, Florida. The disease occurs on all aboveground parts of the host plants, but is most destructive on the foliage. Leaf blades are killed from the point of attachment of petiole and blade as the fungus advances over them in typical thread-blight fashion from the stem and petiole or from an invaded part to a noninvaded part when there is contact. The hyphae apparently are both subcuticular and superficial. The former invades the host cells, killing them; the latter spreads over the leaf-blade surfaces. The killed areas first appear scalded, then dry out, often becoming brown. Hosts found infected are the Bountiful variety of string beans, Fordhook Lima beans, cockle bur, *Xanthium americanum*, and groundnut, *Glycine apios*. This disease is very similar to that described on fig and caused by *Rhizoctonia microsclerotia*. It and a blight disease of Japanese varnish tree, *Firmiana simplex*, are probably identical. The sclerotia produced in nature on bean and fig are quite comparable. The sclerotia produced in cultures by the fungus from the bean are larger and more irregular than those produced on the host.

A Fungus Spot of Azalea Flowers. FREEMAN WEISS.

In 1931, flowers of cultivated azaleas, *Azalea indica*, near Charleston, S. C., were devastated by a hitherto unknown disease. Within a few days of its first appearance flowers by thousands became spotted, then collapsed. The disease has since recurred annually, always coincident with the period of maximum bloom, though varying from late February to mid-April. Only flowers are affected; from adjacent buds healthy leaves emerge and the bushes exhibit no general malaise. Among various organisms isolated from affected flowers (Alternaria, Botrytis, Cladosporium, Pestalotia, yeasts, bacteria) a fungus of exceptional morphology was found which proved upon experimental inoculation solely responsible for the characteristic features of the disease. Within the flower tissue this fungus produces a coarse, septate mycelium in whose train the cells are thoroughly disorganized. Conidia suggestive of Ovularia are first produced, then microconidia typical of Sclerotinia. Subsequently cupulate, smooth, black sclerotia are formed. Microconidia and sclerotia, but not conidia, form readily also on artificial media. Indications of heterothallism and of immature iscomycetous fruit bodies in cultures have been observed. The anticipated rôle of the flower sclerotia has not proved susceptible of experimental proof, outbreaks of disease occurring where they are only a distant factor. The agency of bees (Apis, Bombus, Xylocopa) in disseminating and inoculating the fungus into flowers has been demonstrated.

Bottom Rot of Calla Rhizomes. FREEMAN WEISS and THELMA B. POST.

The rhizome of *Zantedeschia aethiopica* is an upright fleshy stem of indeterminate apical growth; its basal portion dies back as the crown expands and ceases to produce

roots or shoot buds. The moribund basal portion typically is not delimited from the vital middle and crown region by an abscission layer, a condition that invites fungous invasion. In the commercial handling of callas in Pacific Coast nurseries, especially in their transportation to eastern markets, extensive spoilage from bottom rot—a combination of physiological and pathological factors—has occurred in recent years. The Godfrey variety is especially subject to this deterioration. Among the commonly associated fungi are Alternaria, *Phyllosticta richardiae*, *Fusarium* spp. (Sect. *Martiella*), *Rhizoctonia solani* and a Phoma (in von Höhnel's sense) not hitherto recorded on this host. Bacterial soft rot has little, if any, part in the complex. *Phytophthora richardiae*, which causes an aggressive rhizome rot when inoculated into wounds, cannot ordinarily be detected in nor isolated from natural cases of bottom rot. The Phoma is definitely capable of wound parasitism. Alternaria and Phyllosticta appear to be only casually associated with infected rhizomes, but both are parasitic on aerial organs. Rhizoctonia and the *Fusarium* spp. studied are not pathogenic to growing plants; their rôle in bottom rot of dormant rhizomes is still undetermined.

Serically Active (Haptenic) Carbohydrates of Genotypes of Neurospora tetrasperma and N. sitophila. HAZEL W. WILCOX and GEORGE K. K. LINK.

Polysaccharides isolated from two genotypically self-sterile, inter-fertile strains of *Neurospora tetrasperma* (obtained from B. O. Dodge) reacted positively in precipitin-ring tests with antiserums engendered in rabbits following injection with saline extracts of powders of these strains. Specific soluble carbohydrates isolated from each of the 8 monoascosporous strains of a single ascus of *N. sitophila* (obtained from Dr. Dodge) reacted positively in precipitin-ring tests with each of the 8 antiserums of these strains. Work is under way to determine whether all or some of these strains of *N. tetrasperma* and of *N. sitophila*, respectively, in addition to their common carbohydrates, possess strain-specific carbohydrates. (This research was aided in part by a grant to the University of Chicago by the Rockefeller Foundation.)

Host-Parasite Relationship in Bean Rust. S. A. WINGARD.

Histological studies of rust-infected bean leaves indicate that bean varieties commonly regarded as being rust resistant are hypersensitive. The leaf tissues of the hypersensitive hosts die when invaded by the rust fungus and thereby automatically destroy the fungus before any spores can be produced. In the case of the susceptible bean varieties, the invaded tissues of the host are not destroyed by the rust fungus; but, on the contrary, are stimulated and preserved at the expense of the uninvaded tissues. The colonies of invaded host cells, together with the invading rust hyphae, form the parasitic units which live at the expense of the surrounding host cells. This symbiotic relationship between rust hyphae and invaded host cells continues until after the sori and spores have been produced and the surrounding tissues are too much impoverished to continue to nourish the parasitic units. The rust hyphae in the last stages of spore production lose their ability to take the stain, as if completely exhausted, whereas the invaded host cells seem to be invigorated, as shown by the production of a green island surrounding the sorus.

Sclerotinia Rot of Pumpkin and Squash. P. A. YOUNG.

Sclerotinia caused fruit rot in 4 varieties of squash and 3 varieties of pumpkin in Bozeman, Montana, last year. The rot appeared in the field and in storage. Most of the rotting spots did not touch the soil. In squash, Sclerotinia caused disc-shape, rapidly enlarging, water-soaked spots, 2 to 10 cm. wide. Exuded droplets of yellow sap

studded the margins of many lesions. White mycelium protruded from the centers of some of them. The squashes became dry mummies, containing many large sclerotia. Sclerotinia caused a rapidly developing soft rot of pumpkins, releasing large amounts of liquid and producing dense masses of white mycelium with many black sclerotia that ranged from 0.5 cm. to 15.5 by 12 by 0.5 cm. in diameter. Sclerotinia decomposed most of the seeds in these rotted cucurbit fruits. Sap, exuding from mechanical wounds, may serve as a culture medium for Sclerotinia ascospores, thus facilitating wound infection. A Sclerotinia was isolated from the naturally infected cucurbit fruits, and produced typical rots when inoculated into wounds in healthy pumpkin and squash fruits; it was reisolated from them. Sclerotia from pumpkins produced typical apothecia and spores of *Sclerotinia sclerotiorum*.

A New Variety of Tilletia tritici in Montana. P. A. YOUNG.

Nontreated Turkey wheat, planted in a 200-acre field in 1931, produced wheat with 49 (range 25 to 95) per cent of the heads bunted. Study during 3 years showed that this bunt is a new variety of *Tilletia tritici*. Soil infestation with it caused 0.2 to 4.9 per cent of bunted heads in 3 varieties of winter wheat in an experimental plot. The characters given below distinguish this new variety from *T. levis* and from the other forms of *T. tritici* that occur in Montana. The chlamydospores of this variety are 13 to 18 microns in diameter, usually have very prominent reticulations, and rarely germinate in water at 12° or 25° C. Most of the bunt balls were hard and solid instead of soft and powdery inside. Bunted plants commonly had more tillers than normal plants. Nearly all of the bunted heads were borne by extremely dwarfed stems only 3 to 18 cm. tall. They were short and resembled normal heads with their wide-spreading awns. Seed inoculation caused no bunt in 6 varieties of spring wheat and 9 varieties of winter wheat, but they caused nearly 1 per cent of bunted heads in 5 other varieties of winter wheat.

Insoluble Copper Compounds as Substitutes for Bordeaux. H. C. YOUNG and J. R. BECKENBACH.

Two years' laboratory and field tests indicate that basic copper sulphate, copper oxi-chloride, copper phosphate, and Coposil have limited value as fungicides. Their major limitation—lack of adhesiveness—can be largely corrected by the use of Bentonite and a specially treated clay called Wyojel. The ordinary clays and stickers, such as Kaolin, Bancroft and Dawson clay, Talc, flour, and Fluxit, increase the adhesiveness of the insoluble coppers about one-half of that of the Bentonite group. The results from field tests on cucurbits indicate excellent promise for these copper compounds. The most effective formula used so far was a 1–5–1; that is, 1 pound of the copper compound, 5 pounds of fluffer or filler, and 1 pound of calcium or lead arsenate. The results from preliminary tests on potatoes and celery indicate that the addition of lime decreased the fungicidal value. All of these insoluble copper compounds caused serious injury and russeting of apples in northern Ohio.

THE LIFE HISTORY OF TAPHRINA DEFORMANS

A. J. Mix[1]

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REVIEW OF LITERATURE

Before presenting the results of recent experiments and observations on the life history of *Taphrina deformans* (Fkl.) Tul.,[2] it will be necessary to give an account of previous investigations. This will involve some discussion of other species, especially as regards occurrence of perennial mycelium.

References to this fungus in the earlier mycological literature are chiefly concerned with its occurrence, morphology, and taxonomic position. If the early mycologists were interested in the life history of species of Taphrina they perhaps believed that new infections were traceable to ascospores. De Bary (3) states that in the case of *Taphrina pruni* (Fkl.) Tul., infection is probably accomplished by "*sprossen*" derived from ascospores, but that the manner of infection is not known. Ráthay (20), after a careful study of the distribution of mycelium of *Taphrina pruni* in stem and leaf, decides that this mycelium is not perennial and that new infections are due to spores. The essential points of Ráthay's observations are: (1) Mycelium could never be found in parts of twigs older than the current season's growth; (2) foliage shoots were observed with only the tip diseased or with more than one diseased portion separated by sound portions; (3) diseased shoots were found arising from axillary buds on twigs that were entirely sound and free from mycelium; (4) mycelium could never be traced from a diseased twig out into the new foliage shoot arising from it, and such new shoots might be either healthy or diseased; (5) it was observed that infected parts of stems invariably died, coincidently with the death of infected leaves.

Ráthay (21), in a subsequent paper, reports perennial mycelium for *Taphrina cerasi* (Fkl.) Sadeb., (*Exoascus wiesneri* Ráthay). He found, in winter, mycelium present in primary and secondary bark, pith, rays, and cambium of every part of the witches' broom from the youngest shoots to those three or more years old. In summer he traced hyphae from the

[1] The writer wishes to acknowledge a grant from the Research Committee of the Graduate School, University of Kansas, by means of which part of the cost of publishing the illustrations accompanying this paper was defrayed.

[2] In previous publications the writer has referred this fungus to *Exoascus deformans* Fkl., following the classification of the Exoascaceae proposed by Sadebeck (27). The characters used by Sadebeck to separate genera seem to be perfectly reliable. Nevertheless, most mycologists do not follow his classification and recognize but one genus, Taphrina.

hymenium on the leaves to the mycelium within the stem. This is the first circumstantial account of perennial mycelium in the Taphrinaceae and it should be emphasized that in this case the host deformation is a perennial structure. Ráthay's findings were confirmed by Kutsomitopulos (13).

Sadebeck (24), as the result of observations on *Taphrina tosquinetii* (West.) Magn. (*Exoascus alnitorquus* (Tul.) Sadeb.) and *T. ulmi* (Fkl.) Sadeb. (*E. ulmi* Fkl.), states that in these forms the mycelium is perennial beneath the cuticle in the buds. In the taxonomic portion of his paper he includes *T. deformans, T. pruni,* and two other species in a group in which the mycelium is perennial in the inner tissues of the twigs. These statements of Sadebeck rest on careful observations in the case of *T. tosquinetii,* but for the other forms mentioned they are pure assumptions.

Johanson (10), after careful study of the distribution of mycelium in the host, decides that *Taphrina tosquinetii, T. betulina* Rostrup, and *T. borealis* Johans. possess perennial mycelium, while *T. carnea* Johans., *T. sadebeckii* Johans., and probably also *T. betulae* Fkl., do not. It is worth considering that these last named species affect only the leaves and stems of the current season's growth and do not form witches' brooms or other malformations on the older parts of the host.

Johanson also suggests that in species of Taphrina, whether possessing perennial mycelium or not, the bud conidia derived from ascospores may overwinter and cause infection the following spring.

Sadebeck (25) disagrees with Johanson as to the lack of perennial mycelium in the species mentioned above but presents no evidence. In a later paper Sadebeck (26) states that in species possessing only subcuticular mycelium this mycelium overwinters in the buds, while in species whose mycelium penetrates deeper into the host it is perennial in the older tissues of the shoots. He repeats his account of observations on *Taphrina tosquinetii,* retracts his former statement that *T. ulmi* has perennial mycelium, and again, in his taxonomic account, divides the forms with perennial mycelium into those in which it overwinters in the buds, and those in which it overwinters in the stems. He places *T. deformans* in the latter group, stating that the mycelium overwinters in the youngest parts of the one-year twigs, and may be found in the primary bark, pith, and rays of the first shoots of each vegetative period, growing out into the leaves and ultimately forming the subcuticular hymenium. He does not report having found mycelium in new stems earlier than in leaves, and it is important to realize that his account of the distribution of mycelium in leaves and stems may be interpreted as indicating that this mycelium has entered the stem from the leaf. In fact, Sadebeck's contributions concerning perennial mycelium in the Taphrinaceae adduce evidence of its occurrence in one species, *T. tosquinetii,* and present ungrounded assumptions of its occurrence in several other species, including *T. deformans.*

Atkinson (2), apparently following Sadebeck, asserts the presence of perennial mycelium in *Taphrina deformans, T. cerasi, T. pruni, T. mirabilis* Atk. and *T. decipiens* Atk. In his general discussion of pruniculous Taphrinaceae Atkinson states . . . "the species possess a perennial mycelium." He says further that the mycelium of *T. deformans* passes the late summer, autumn, and winter in the tissue of the leaf buds, growing out in the spring into the developing leaves, and believes that this explains the fact that buds taken from affected trees transmit the disease to nursery trees. These statements of Atkinson rest neither on cited authority (except in the case of *T. cerasi*) nor on recorded observations.

Duggar (6) points out that, occasionally, twigs affected by peach leaf curl do not die but make new healthy growth above the swollen diseased portion. He shows a photograph of twigs that have thus outgrown the disease and states that the fungus overwinters in the swollen diseased parts of such twigs. He does not report having found this overwintering mycelium. Duggar suggests two other possible ways in which the fungus may survive: (1) By spores infecting the buds immediately after dispersal, (2) by spores overwintering on the twigs, in the bud scales, or on the ground, and infecting the opening buds in the spring. His spraying experiments support the latter idea, protection being secured when trees were sprayed before the buds began to swell but none from spraying after the leaves emerged.

The spraying experiments of Pierce (19) show that infection by overwintering spores must occur, and Pierce believes that these are responsible for nearly if not all curl. Since a single spraying gave 97 to 98 per cent control, only 2 or 3 per cent of the curl of a given season could possibly be due to overwintering mycelium. Pierce points out that Sadebeck gives no account of experimentation to support his assertion that the mycelium of *Taphrina deformans* is perennial. Pierce's own studies of the distribution of mycelium in diseased twigs lead him to believe that mycelium enters the twig from the leaf, especially as it diminishes in amount with distance from the diseased petiole.[3]

Recently Fitzpatrick (8), by means of spraying experiments has determined closely the time of infection in two seasons in eastern Ontario, and has correlated these infections with rain periods. He concludes that leaves are susceptible when emerging from the bud and for a short time thereafter, a fact that has been fully recognized since the spraying experiments of Duggar and Pierce.

Following Pierce's work most pathologists have accepted the idea that infection is due to overwintering spores lodged on the buds or twigs and

[3] Pierce cites a number of earlier authors, such as Frank and Sorauer, who assume that mycelium of *Taphrina deformans* is perennial. These citations are omitted from the present account, since they offer no experimental proof.

that perennial mycelium, if present, can be responsible for only a small proportion of curl. However, the exact manner of infection by spores has been in doubt.

The writer's earlier work (15) proved that conidia derived from ascospores by budding may infect nascent leaves and even those that are out of the bud and considerably enlarged. The natural occurrence of late infection on large leaves is reported in a subsequent paper (16).

The writer has further shown (15) that conidia are capable of withstanding desiccation for periods long enough to explain their survival from the time of ascospore dispersion until that of infection. That survival in nature is aided by a saprophytic multiplication of conidia is a reasonable assumption. The fungus thrives in culture in a wide variety of media, many of them of low food content, as, for example, a very dilute solution of peach gum, and it is difficult to believe that it may not find occasional opportunity for saprophytic growth in solutions naturally occurring on peach twigs. There should, in fact, be no more mystery about the survival from one season to the next of the saprophytic bud conidia of *Taphrina deformans* than there is about that of yeast cells.

ATTEMPTED ISOLATIONS FROM TWIG SURFACES

It would be gratifying if direct evidence of the presence of overwintering conidia on peach twigs could be obtained by means of isolations from twig surfaces. A number of unsuccessful isolations have been previously reported (15). An attempt has since been made to recover the fungus from twig surfaces when it was known to be there. On Nov. 2 and 29, 1926, several branches of peach trees were sprayed with spore suspensions of *Taphrina deformans* and plate isolations were made on Nov. 30, Jan. 6, and Feb. 24. Numerous colonies of both budding and filamentous fungi developed in these plates and many transfers were made from the budding colonies. In this manner 4 cultures of *T. deformans* were obtained, all from isolations made on Nov. 30. None was secured from the later isolations. That the fungus did, however, survive on these twigs is indicated by counts made on April 28, which showed, on the inoculated branches, 165 curled shoots and 132 healthy, while on branches subject only to natural infection (selected at random but avoiding those close to the inoculated branches) 20 curled and 580 healthy shoots were found.

The difficulty attending these attempted isolations is, as already pointed out (15), due to the occurrence on twig surfaces of budding and filamentous fungi all of which grow faster in culture than does *Taphrina deformans*. It does not seem possible to obtain a differential medium for this fungus. On any medium that has been found to support its growth, saprophytic forms grow as well or better.

Since the curl fungus grows (15) in highly acid media, isolations from twig surfaces were attempted on acidified potato-dextrose agar (pH 3.5). This also allowed growth of many saprophytic forms and the curl fungus was not obtained.

The procedure in these isolations was varied by distributing a few cubic centimeters of twig washings in a large volume of medium (500 to 1000 cc.) and using all the medium in pouring plates. This resulted in less crowded plates but did not allow isolations of the curl fungus. Fitzpatrick (8) seems to have succeeded where the writer has consistently failed.

INOCULATION EXPERIMENTS

Successful inoculation experiments in which cells from pure cultures of *Taphrina deformans* were used have previously been reported (15). Since no facilities were at hand for keeping potted trees in the greenhouse under conditions sufficiently cool and humid to insure infection, these inoculations and those to be reported below were performed on trees growing out of doors. Young Elberta trees were obtained from a nursery in the spring of 1922 and planted on the university campus. The nearest peach tree was over a quarter of a mile distant and there were no peach orchards within a mile. Certain branches on these trees were sprayed with Formalin 1–200 and subsequently inoculated in the spring of 1922 and again in 1923 (15). The curled leaves were carefully removed and destroyed each season before ascospores were formed. The trees were not inoculated in 1924 or 1925 and there was no curl on them in those years. It was, therefore, thought safe to omit the spraying with Formalin in the following inoculation experiments. A few branches on these trees were inoculated in October, 1925, and in January, March, and April, 1926, but no curl resulted. These were very unfavorable seasons for curl, as will be explained more fully below.

In 1926–27 a large number of branches were inoculated, in July, November, January, February, March, and April. Two methods were used, (1) smearing the buds with the yeast-like mass of cells obtained in culture, (2) spraying twigs and buds with a suspension of cells in sterilized water. Two cultures of *Taphrina deformans* were used, one (No. 11), isolated in 1920 and the other (No. 101), isolated in 1926. For smear inoculations these cultures were used alternately; in the spray inoculations they were mixed.

In making the counts no attention was paid to severity of infection, a shoot being counted as "curled" whether there was one curled leaf or many. In most cases, however, several leaves of each shoot showed curl.

The results (Table 1) indicate that conidia from culture may survive on the twigs from mid-July (approximately one month after ascospore dispersion) to the following spring, and still cause infection. While most

TABLE 1.—*Results of inoculations of peach branches with Taphrina deformans, 1926–27*

Branch	Date	Method	Culture	Curled shoots	Healthy shoots
97	July 13, 1926	Spray	11 and 101	29	8
2	Nov. 2, "	"	" " "	27	2
77	" " "	"	" " "	83	19
23	" " "	Smear	11	58	11
94	" " "	Spray	11 and 101	117	23
40	" " "	Smear	101	46	0
34	" 29 "	Spray	11 and 101	135	62
47	" " "	"	" " "	46	27
58	" 30 "	Smear	11	13	3
59	" " "	"	" " "	17	1
38	" " "	"	" " "	13	3
18	" " "	"	" " "	16	2
26	" " "	"	101	24	3
3	" " "	"	"	18	0
6	" " "	"	"	10	0
19	Jan. 5, 1927	Spray	11 and 101	25	30
32	" " "	"	" " "	10	17
17	" " "	Smear	101	10	25
83	" " "	"	11	1	10
7	" " "	"	"	12	8
80	" " "	"	"	19	8
46	Feb. 22, "	Spray	11 and 101	100	35
4	" " "	Smear	11	22	39
85	" " "	"	"	29	10
62	" " "	"	101	22	15
56	Mar. 9, "	"	11	63	35
79	" " "	"	101	25	6
8	" 15, "	"	"	3	24
29	" " "	"	11	7	40
13	" 19, "	Spray	11 and 101	175	125
5	" 31, "	"	"	36	157
		Total		1211	748
		Percentage curl		62	
		Control branches near inoculated branches		84	260
		Percentage curl		21	
		Control branches remote from inoculated branches		95	730
		Percentage curl		11	

of the inoculations of this season were made on branches not inoculated the previous year, branch number 40 was inoculated on Feb. 23, 1926, and again on Nov. 2, 1926. It will be noticed (Table 1) that every shoot on this branch became infected in 1927. Facts not shown in the table are

that every leaf of every shoot was curled, and many shoots showed distortion of the twigs by the curl fungus. The unusual severity of the curl on this branch may well be explained by the larger amount of inoculum, and may also be taken to indicate that conidia from culture survived on the twigs from February, 1926, until the spring of 1927.

The occurrence of curl on the control (noninoculated) branches of these trees may be taken as further evidence of the survival of conidia for more than one year. The remoteness of these trees from those affected by curl, and the near absence of natural curl in this locality in 1926, suggest that the curl on these noninoculated branches in 1927 was due to inoculations in the preceding year. Even if it is considered to be due to natural inoculation, survival of conidia for more than one year is, nevertheless indicated, since there was no curl whatever on these trees in 1926.

In the spring of 1929 peach seedlings bearing dormant Elberta buds were obtained and planted in pots in the greenhouse. The seedling tops were cut off and the stumps sprayed with Formalin 1–200. After the new Elberta shoots had made considerable growth the trees were again sprayed with Formalin and, on July 9, a few days later, were inoculated by the spray method. These trees were kept in the greenhouse until early September, when they were planted outside. Counts of curled shoots (Table 2),

TABLE 2.—*Results obtained from potted trees inoculated with Taphrina deformans. The inoculations were made on July 9, 1929, and the results were recorded on May 15, 1930*

Tree No.	Culture	Curled shoots	Healthy shoots
1	4	13	17
2	C1	12	16
3	C7	14	16
4	1	32	44
5	2	28	16
6	3	30	24
7	Control	0	42

made in May, 1930, indicate that conidia had survived on these trees from July until the following spring. Wieben (30) reports inoculating peach buds with pure cultures of *Taphrina deformans* in the summer, with infection resulting the following spring.

Bagging Experiment

On April 30, 1927, a number of peach shoots were enclosed in transparent, waterproof paper bags. The shoots chosen were free from curl.

Nearby shoots bore many curled leaves approaching the stage of ascospore dispersion. Ejection of ascospores began about May 2 and was nearly finished by May 20. The bags were left on until June 11, when all curled leaves had fallen. Bags on branches 13 and 19 were left in place during the summer and were finally torn open by wind and rain in the fall. The results of the bagging experiment (Table 3) indicate that when spores from

TABLE 3.—*Results of bagging peach shoots on trees infected with curl*

Shoot No.	Date of bagging	Bag removed	(1928) Curled shoots	(1928) Healthy shoots
23	April 30, 1927	June 11, 1927	0	6
2	" " "	" " "	1	5
21	" " "	" " "	0	5
6	" " "	" " "	0	12
18	" " "	" " "	Shoot dead	—
9	" " "	" " "	0	13
19	" " "	Not removed	0	9
13	" " "	" "	Shoot dead	—
Controls—Shoots chosen at random in these trees in the spring of 1928			55	630

curled leaves are prevented from reaching peach twigs, curl does not result the following spring.

SUMMER SPRAYING

Reddick and Toan (22) have shown that fall spraying effectively prevents curl. It seemed possible that summer spraying also might prove effective, and furnish additional evidence of the spores on twig surfaces. Facilities were not at hand for spraying on a field scale but a few branches of affected trees were sprayed with lime-sulphur 1–100 on May 19, 1927 (during the period of ascospore dispersion), and others on June 25 and June 27 (after ascospore dispersion had ceased). Counts made in the spring of 1928 showed 21 curled shoots to 290 healthy (6.8 per cent curl), but counts of shoots chosen at random on unsprayed branches showed 23 curled shoots to 315 healthy (6.9 per cent curl). The experiment was quite inconclusive, and, because of the difficulty in spraying the twigs thoroughly when there is an abundance of foliage, no further experiments were attempted.

FIELD OBSERVATIONS

The development of peach leaf curl in eastern Kansas in 1924 has been previously described (16). Infection occurred only after the leaves were partly grown and the total amount of curl was much smaller than usual.

In 1925, although there was a prolonged rain when the peach buds were pushing and two later rain periods occurred, there was not so much wet spring weather as is usual in this locality. Curl was more abundant than in 1924 but much less abundant than in most seasons.

In 1926 there was no prolonged rain during the period of susceptibility. Inoculation experiments made this spring failed (as noted above) and only a very few curled leaves could be found in orchards usually badly infested.

The occurrence of curl in 1927 has been briefly described elsewhere (16). Following two years of scarcity of curl, and a third year in which it was almost entirely absent, the outbreak in 1927 was the most severe during the ten-year period of the writer's observation. Rain periods of six or more hours' duration occurred on March 10 (peach buds showing green tips), March 19, (leaves $\frac{1}{4}$ inch out of buds), March 19–20, March 30–31, April 6–7, 8, 12, and 13–14. Curl in its early stages was very prevalent by April 15. On April 27 orchards in this vicinity showed 95 per cent curl, *i.e.*, 95 per cent of the season's shoots showed curled leaves. This does not give a true picture of the severity of the disease, since in nearly every case every leaf of a shoot was curled.

It does not seem possible that the very few curled leaves in these orchards in 1926 could have produced ascospores and conidia sufficient to account for such an unprecedented amount of curl. The explanation must be that conidia are able to survive on twig surfaces longer than one year. This need not imply survival of individual conidia for so long a period, as saprophytic multiplication of conidia is quite possible.

Similar observations are reported by Young (32) and Anderson (1). Young describes an unusually severe outbreak of curl in Arkansas in 1926, following its marked rarity in 1925. Anderson describes the epidemic in Southern Illinois in 1926 as the worst he had ever seen, and states that curl had been almost completely absent there in the few preceding years.

Occurrence of Late Infections

The behavior of curl during a season following late spring infection has been described (16) as well as the fact that lesions due to late infection are small, separate areas scattered over the leaf blade. Such lesions are observable in many seasons when early infections also have occurred but are not always recognized.

Further evidence of late infections is obtained from observations of localized lesions on leaves some distance above the base of the season's shoot. The peach has indeterminate growth and not all the leaves of a season's shoot are present when the bud unfolds. There are usually 6 or 7 leaves present within the dormant bud. Small curl lesions on the blade of the leaf with no evidence of the disease on the petiole or adjacent part of the

shoot have often been observed on leaves arising from the ninth, tenth, eleventh, and twelfth nodes from the base of the shoot.

Waite (28) recently has described the occurrence in Alabama of an early curl infection involving the oldest leaves of a shoot and a later infection producing younger lesions on the tip leaves of shoots a foot or so long with from 7 to 11 healthy leaves below. Waite discusses the possibility of this late curl being due to secondary infection, but inclines rather to the belief that it may have been a case of delayed dormancy: *i.e.*, the fungus had not, because of an unusually mild winter, experienced enough cold to prepare it for spring germination. There is nothing in the life history of *Taphrina deformans* to suggest the necessity of a dormant period. In the experiments reported above infection resulted from inoculations made at intervals during the summer, fall, winter and spring. Inoculations of leaves just emerging from the buds with conidia that had been budding actively in culture for a year or more were quite as successful as those made earlier. The fungus clearly needs no rest period preliminary to infection.

It is probable that the early and late infections described by Waite could be definitely correlated with rain periods.

Secondary infections by the curl fungus may occasionally occur. Small, scattered lesions typical of late infections may sometimes be found on leaves so many internodes distant from the base of the twig that these leaves must have formed and become infected after ascospore dispersion from earlier infections. Such leaves are less likely to fall prematurely than those less recently infected. One such leaf has been observed on the tree as late as September.

The occurrence of late infections, whether primary or secondary, is of little practical significance. Spraying for curl is clearly an eradication measure, resulting in the destruction of conidia present on twig and bud surfaces, and if carefully done, it controls the disease. But growers may perhaps be made to realize that when their trees have escaped early infection there is still danger of curl that same season, and may thus be induced to spray every year.

PLACE OF HIBERNATION OF CONIDIA

The experimental results and observations reported here and earlier (15, 16, 17) together with the results of spraying experiments of Duggar (6), Pierce (19), and others point to the conclusion that bud conidia of *Taphrina deformans* pass the summer and winter on the surfaces of peach twigs and branches. Waite (28) states: "The spores . . . are supposed to lodge in the buds in late spring and early summer and to rest among the hairs in the bud scales during the winter. . . ." Wallace and Whetzel (29) state that circumstantial evidence indicates that spores are harbored

through the winter most probably on or between the bud scales. The same opinion is expressed elsewhere, as, for example, in Duggar's textbook: Fungous Diseases of Plants.

That the bud scales of the peach harbor a number of budding fungi has been shown by the writer (15), and the bud pubescence undoubtedly aids in retaining spores on bud scales once they have lodged there. At the time of ascospore dispersion, however, there are no peach buds with scales; only bud fundaments covered by the base of the petiole. Further, the bud conidia adhere to smooth surfaces. Cells from culture allowed to dry on glass wash away gradually under a spray of water, but some remain even after several hours' exposure.

There seems to be no reason for excluding any part of the surface of the peach tree as a place of hibernation.

Perennial Mycelium

Although it is clear that spring infection is traceable to overwintered bud conidia, this fact does not preclude the possible occurrence of perennial mycelium. Isolations from diseased twigs, undertaken to determine how long the mycelium survives in them, have already been reported (15), and additional isolations are described later in this paper. Success in such isolations does not depend, as was at first supposed, upon growth of mycelium from the diseased twig onto the agar. The method is, therefore, of no value in determining the point in question.

It becomes necessary, then, to rely on the observed occurrence of mycelium in twigs that have survived the disease and have made healthy growth beyond the hypertrophied portion, as described and figured by Duggar (6). Such twigs occur rarely in this locality and only on certain varieties. A few trees bearing them have been kept under observation and careful examination of the twigs was made in 1929, a season in which curl was early and was confined to the oldest leaves of the season's shoot.

In all cases the curl lesions involved the leaf only, sections failing to reveal mycelium in the stem of either the previous or the current year or even in the petiole. On the other hand, many curled shoots of the current year from branches that were healthy the year before showed hypertrophy of the petiole and adjacent portions of the stem. In such cases mycelium could be traced from the leaf through the petiole and down the stem to its point of disappearance. In no case did it extend more than a short distance down the twig of the current year. It is clear that, as in the curled shoots studied by Pierce (19), the mycelium had entered the stem from the leaf. A number of twigs that, this season, made healthy growth beyond the hypertrophied portion were sectioned and examined. A supplementary study was made of similar twigs collected in previous years and preserved

in Formalin or alcohol. Not only was the mycelium of *Taphrina deformans* absent from these twigs, but all the tissues formerly occupied by the fungus were dead. Healthy growth, resulting in extension of the twig, had been accomplished by tissues never occupied by the fungus.

From the peculiarities of *Taphrina deformans* when growing in culture, it is inconceivable that its mycelium can remain alive in dead host tissues, and the absence of mycelium from adjacent healthy tissues indicates the nonexistence of perennial mycelium. It has been shown earlier in this paper that the belief of various writers that *T. deformans* possesses perennial mycelium is based either on pure assumption, or on the imperfect observations of Sadebeck (25, 26). Unfortunately, the mere announcement of this belief has placed a burden of disproof upon subsequent investigators. It is proposed here to shift this burden and to state that, since there is no evidence whatever of the existence of perennial mycelium in *T. deformans*, it does not exist.

Complete study of the Taphrinaceae will undoubtedly reveal three types: forms with mycelium perennial in the branches, causing witches' brooms (*Taphrina cerasi*); forms with perennial mycelium in the buds, deforming whole shoot systems of the current season (*T. tosquinetii*); forms without perennial mycelium, affecting leaves (*T. aurea*); or leaves, fruits, and adjacent parts of twigs (*T. deformans, T. mirabilis*).

THE FUNGUS IN THE HOST: PENETRATION

Entrance of the fungus into the host has been observed in a few cases when conidia from culture were placed on detached leaves kept in moist Petri dishes. Portions of the leaf blades were then cleared and mounted. Other instances of penetration have been seen in fixed and stained sections of naturally infected leaves taken at frequent intervals, after the occurrence of prolonged spring rains, from heavily infected trees.

In all cases observed the fungus entered through the lower epidermis. Entrance through the upper epidermis, as reported by Fitzpatrick (8), undoubtedly occurs in the case of late infections but until the young leaf is well out of the bud it is folded along its midrib and the upper surfaces of the two halves are so held together by a waxy material that some difficulty is experienced in separating them. Only the lower surface of the leaf, therefore, is exposed.

The germ tube penetrates the epidermal wall (Fig. 1, A). Martin (14) states that she observed stomatal entrance, but the length of time (25 days) between inoculation and penetration, the fact that infection did not occur, and the lack of resemblance of the hyphae (figured approaching (not entering) the stoma) to mycelium of *Taphrina deformans* make her observation doubtful. Stomatal penetration may perhaps occur in late infections, but

in the infections of young leaves observed by the writer, it was impossible because the stomata were not yet developed. In sowings on older, detached leaves, spores often have been seen lodged in the stomatal entrance, but the growth of a germ tube into the stoma has not been observed.

In the youngest leaves in which penetration was observed there was no demonstrable cutinization of the epidermal wall, except directly beneath a large vein. The epidermal cells, however, contained numerous globules of a fatty material. In somewhat older leaves the fungus was seen penetrating a thinly cutinized epidermis. To what degree cutinization of the epidermal wall and the development of cuticle may act as a barrier to infection remains to be determined.

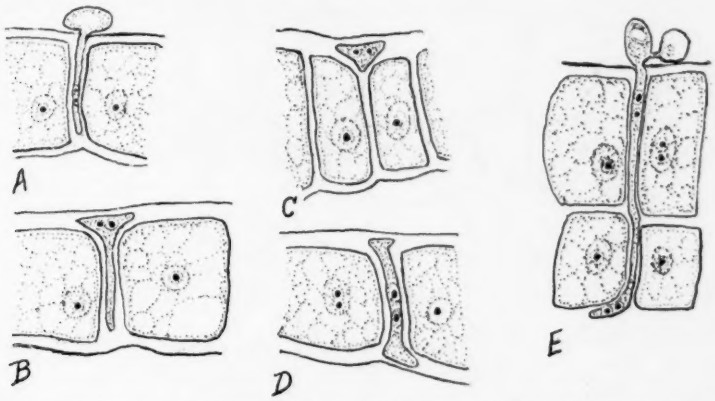

FIG. 1. Stages in penetration of the leaf by *Taphrina deformans*. A. Entrance hypha has penetrated epidermal wall and is growing in the radial wall between two cells of the epidermis. C. Subcuticular hypha, the spore is not visible. B, D. Subsequent growth of subcuticular hyphae in radial wall of epidermis. E. A case of penetration in which copulation of conidia apparently had preceded penetration.

After passing through the epidermal wall the penetration hypha often grows laterally, as reported by Fitzpatrick (8). Thus, stages in which the hypha is visible but not the spore itself are commonly seen (Fig. 1, C). The hypha now grows down between two adjacent epidermal cells (Fig. 1, B, D). This growth takes place perforce in the wall between the two cells. No intercellular spaces occur between the radial walls of the peach epidermis. Fitzpatrick (8), whose observations on penetration agree in general with those of the writer, is silent on this point, but one of his illustrations seems to show intercellular spaces between epidermal cells.

In all but one of observed penetrations the entrance hypha had arisen from a single conidium. In one case, however, (Fig. 1, E) copulation of conidia had apparently preceded emergence of the germ tube. One other instance of apparent copulation and subsequent growth of a tube from one

of the conidia has been seen on the surface of a cleared leaf; penetration, however, had not occurred. In the light of the findings presented below these observations are unusual.

SUBSEQUENT COURSE OF MYCELIUM

From the entrance hypha the mycelium extends at first (Fig. 2, A) between the lower epidermis and the first hypodermal layer (the spongy parenchyma not being yet differentiated). Subsequently, hyphae spread throughout the leaf, growing between the interior mesophyll cells. On reaching the upper epidermis, they spread out beneath it. This subepidermal mycelium is more abundantly developed than that beneath the

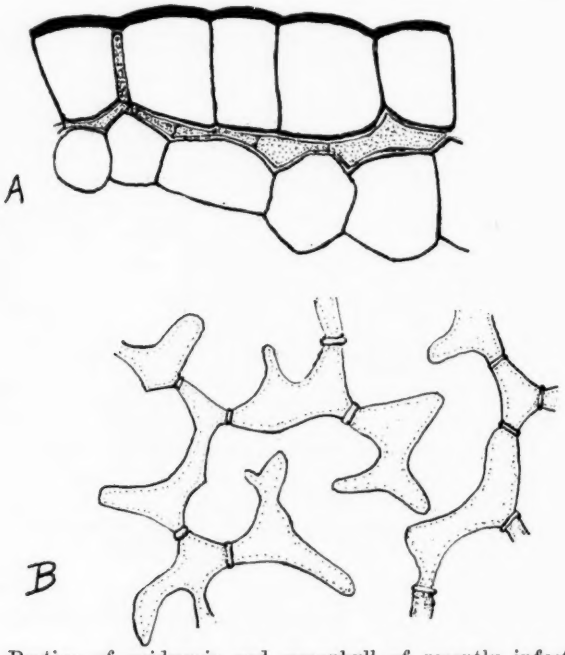

FIG. 2. A. Portion of epidermis and mesophyll of recently infected peach leaf, showing the subepidermal mycelium. B. Subepidermal mycelium as viewed from above in a cleared leaf. Host cells not shown.

lower epidermis. When viewed from above in cleared leaves, it appears as shown in figure 2, B. The irregular shape of these hyphae is apparently due to their fitting closely the intercellular spaces. The plate septa described by Pierce (19) may frequently be seen (Fig. 2, B). Judging from the appearance of these plate septa and from their observed swelling and dissolution in hyphae formed in culture they seem to arise as thickenings of the wall and to function as separation discs, allowing adjacent cells of the mycelium to separate from each other.

Frequently, in older much-curled leaves, the well-known tissue modifications are found only in the upper portions, involving upper epidermis, palisade parenchyma, and part of the spongy parenchyma, while the lowermost parenchyma and lower epidermis remain healthy and apparently free from mycelium. This suggests the possibility of entrance of the fungus through the upper epidermis, though it might conceivably result from the course of events described above together with more abundant development of the fungus in the upper part of the leaf.

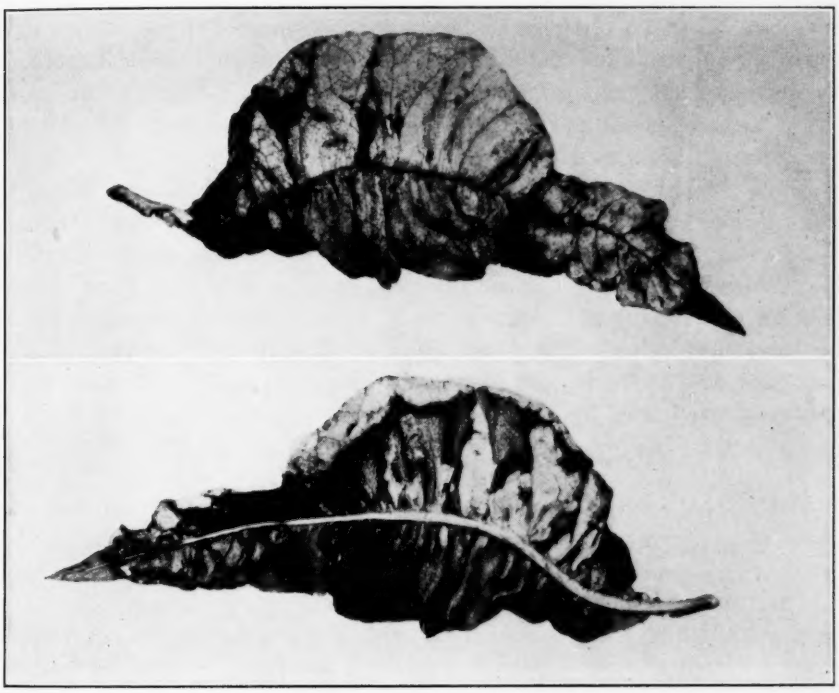

FIG. 3. A curled peach leaf that bore asci of *Taphrina deformans* on both its upper and lower surface. The whitish areas show the location of the hymenium.

From the mycelium beneath the upper epidermis, hyphae grow upward in radial epidermal walls. Branches of these hyphae form the subcuticular mycelium, ascogenous cells, and asci. A point of minor importance is the occasional occurrence of the hymenium beneath the cuticle of the lower epidermis. Winter (31) states that the asci break through the lower surface of the leaf, and this statement is denied by Knowles (12). Robinson (23) states that asci occur on both surface of the leaf but in greater numbers on the lower surface; Atkinson (2) finds them on both surfaces; while Pierce (19) dismisses these statements as having arisen "from a study of

other foliage than that of the peach and of other species of *Exoascus*. . . ." Asci do sometimes occur on both surfaces of the leaf in the case of badly curled leaves of susceptible varieties (Fig. 3).

CYTOLOGY

According to Dangeard (4), cells of the vegetative hyphae of *Taphrina deformans* are binucleate. Ikeno (9) describes them as multinucleate, Eftimiu (7) as binucleate or plurinucleate (12 to 14 nuclei). Eftimiu describes conjugate division of these nuclei. Fusion of the two nuclei in an ascogenous cell is reported by Dangeard (4), Ikeno (9) and Eftimiu (7). According to Ikeno and Eftimiu, the fusion nucleus divides by 3 successive divisions to form the nuclei for the 8 ascospores. Eftimiu identifies the first of these divisions as a reduction division, and reports the diploid number of chromosomes as 4, the haploid as 2.

Since the conidia are uninucleate, the manner in which the cells of the intercellular mycelium become binucleate has been uncertain. Eftimiu (7), having found binucleate conidia and germ tubes, decides that this condition is reached by nuclear division. Fitzpatrick (8), from observations on penetrating germ tubes and of tubes formed in culture, reaches the same conclusion. The penetration hyphae described above show in some cases one or more pairs of nuclei (Fig. 1, A, B, C, D), which apparently have originated by division of a single conidium nucleus.

COPULATION OF CONIDIA

Wieben (31) describes copulation of conidia and differentiation of the spores of one ascus into two sexes for *Taphrina epiphylla* Sadeb. and *T. klebahni* Wieben. The nucleus migrates from one conidium to the other. This binucleate conidium germinates by a long spiral thread. The tip of this thread, which may be cut off by septa from the older empty portions, contains cytoplasm and a pair of nuclei. Presumably this is the infection thread, and copulation of sexually different conidia may be a necessary preliminary to infection. Wieben's investigations, however, were not extended to determine this.[4]

In order to learn whether copulation of conidia may occur in the case of *Taphrina deformans*, cells from culture were placed on hanging drops of agar and observed from day to day under the microscope. In a few cases adjacent conidia put forth short papillae that joined to form a tube and one of the two conidia became progressively more vacuolate. Complete

[4] During a stay in Hamburg in 1930 the writer obtained a number of monosporous cultures of these fungi, but before the host (*Alnus incana*), which does not occur in Kansas, could be grown large enough for inoculation experiments, these cultures died from accidental exposure to high temperature. For the present this interesting question cannot be answered.

passage of the contents of one conidium into the other was not observed. In stained preparations cases were found showing apparent passage of a nucleus from one conidium to another and the subsequent emergence of a tube from the second conidium (Fig. 4). From other conidia long twisted

FIG. 4. Cases of apparent copulation of conidia seen in stained preparations from cultures of *Taphrina deformans*.

tubes bearing one or more pairs of nuclei were seen to arise (Fig. 5, F, G). These tubes, however, arose from single conidia, and it could not be determined whether copulation had preceded their formation.

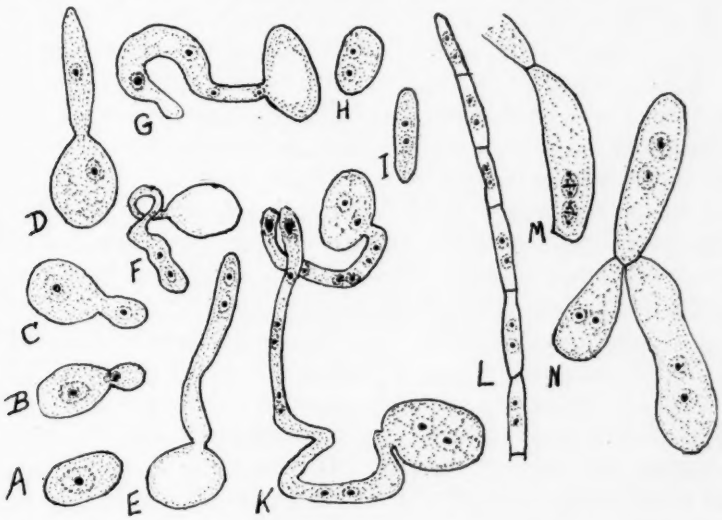

FIG. 5. Types of cells formed by *Taphrina deformans* in culture. A, B, C. Budding conidia. D. Conidium with straight, uninucleate tube. E, F, G. Conidia with irregular tubes bearing one or more pairs of nuclei. H, I. Binucleate conidia. K. Conidia with plurinucleate tubes joined at their tips. L, M, N. Hyphae with binucleate cells. M. Conjugate division in a binucleate hypha.

These observations were reported before the American Phytopathological Society in December 1928. In the abstract (18) of that paper it was stated for the sake of brevity that copulating conidia and binucleate germ tubes had been observed. In the light of subsequent findings it is regrettable that this statement was not fuller and more accurate.

The following summer a series of 8 monosporous cultures from a single ascus of *Taphrina deformans* was obtained. Asci were allowed to eject

their spores onto a film of agar on a cover slip.[5] An isolated group of 8 ascospores was chosen and the individual spores were separated by the Dickinson (5) method, using a micromanipulator devised for the purpose. From the resulting colonies transfers were made to agar slants and somewhat later cells from these cultures were placed on agar films and observed under the microscope. All possible pairings between the 8 cultures were made. Copulation observed was only occasional and incomplete (as described above) and there was no indication of sexual differentiation between the cells of different monosporous cultures. The experiment was repeated many times, using a number of different media, with no other results.

In the summer of 1930 the writer was privileged to work in Dr. Klebahn's laboratory in Hamburg and to repeat, with the assistance of Drs. Klebahn and Wieben, a part of the latter's work with *Taphrina epiphylla* and *T. klebahni*. Copulation of spores of these fungi was found to occur readily and exactly as described by Wieben (30). Difficulty was encountered in separating the 8 spores of an ascus because of the ejection of a quantity of sticky epiplasm along with the spores. Several incomplete series of 5, 6, or 7 monosporous cultures were obtained, but from lack of time no experiments were made on sexual differentiation.

Since, in the case of the fungi just mentioned, copulation is easily observed in the young colonies developing on agar films around a group of ejected spores, examination was made of such colonies of *Taphrina deformans* in this and subsequent seasons, but without result.

In *Taphrina epiphylla*, copulation occurs soon after conidia are formed, perhaps because infection occurs at once, and no such phenomenon can be observed in old single-ascus cultures. Further, when pairings were made in the fall of 1930 between cells from monosporous cultures some months old no copulation could be observed. On the other hand, the imperfect fusion of conidia observed in *T. deformans* has been found in only comparatively old cultures.

Further study of stained slides made from cultures of *Taphrina deformans* showed that the long twisted tubes arising from conidia are often uninucleate, or if they contain paired nuclei, they bear a single bright-staining nucleus close to the tip of the tube. Several cases were observed in which two such tubes from different conidia apparently were joined at their tips (Fig. 5, K). This suggested that copulation between conidia might have

[5] This method, or one in which the asci eject their spores upward onto agar in an inverted Petri dish, is the usual procedure in obtaining pure cultures of species of Taphrina. The 8 spores of an ascus form a close symmetrical group on the agar, easily located under the low power of the microscope. An ascus group is marked, the colony resulting from budding watched from day to day, and finally, tube cultures are made by transfer. Thus all cultures herein reported, except those from single spores, are from single asci.

occurred by means of such tubes. After much observation of conidia in agar films, one case was finally found where two such tubes came together at their tips and joined. Although kept under observation for several days there was no migration of protoplasm from one tube to the other nor any apparent alteration in the walls at the point of union. At the end of the period of observation the agar film was inverted, a cover slip added and pressure applied. The joined tubes could not be separated by pressure less than that necessary to crush the cells.

One example of apparent union of long tubes from conidia has been observed on the surface of a cleared leaf that had been inoculated and held in a Petri-dish moist chamber.

Inoculation Experiments with Monosporous and Single-ascus Cultures

The above observations indicated that copulation of conidia, as has been found in *Taphrina epiphylla,* does not occur with any regularity in *T. deformans.* However, the case of apparent copulation preceding penetration described in the foregoing, suggested that such copulation occasionally occurs. There was also the further possibility that union between germ tubes from conidia of opposite sex may occur after penetration of the leaf and that such union may be necessary to successful infection or to the formation of ascus-bearing mycelium. It was apparent that this possibility could be determined by inoculation experiments.

The first experiment was performed in 1930 in the Chelsea Physic Garden, London, on two potted yearling Peregrine peach trees furnished by the Department of Botany of the Royal College of Science. Inoculum from culture was inserted into the opening buds, and the high humidity of the greenhouse (an unheated fern house) made it possible to keep the buds wet for several hours. On April 1 one of these trees was inoculated with a single-ascus culture (163), the other with a monosporous culture (D4). On May 16 the first tree showed 20 curled shoots and 43 healthy. The second tree was dying, though it bore 30 shoots with leaves that were large enough to show curl. None of these leaves was curled.

Because of the inconclusive nature of this experiment inoculations were made in April, 1931, on young Elberta trees growing out of doors. These trees had been used for inoculation in the summer of 1929, but curled leaves borne in 1930 had been removed before ascospores were produced. The trees were sprayed with Formalin 1 to 100 a few days before inoculation. Shoots chosen for inoculation were some distance apart and so placed that the danger of splashing of inoculum from one to another was very slight. The fact that curl developed only on the inoculated shoots and on a few noninoculated ones close to some of them indicates that the experiment was trustworthy. Inoculations were made by the "smear method" at the begin-

ning of a rain period. Cultures used were Nos. D1 to D8, a series of monosporous cultures from one ascus, and Nos. 11, 176, 192, single-ascus cultures. Different shoots were inoculated with each monosporous culture, with each monosporous culture paired with every other, and with each single-ascus culture. Counts on May 18 (Table 4) showed curl resulting from every inoculation. All curled leaves produced ascospores.

TABLE 4.—*Results of inoculating peach shoots with monosporous cultures (D1 to D8) from one ascus, and with single-ascus cultures (11, 176, 192) of Taphrina deformans*

Branch number	Culture	Curled shoots	Healthy shoots
1	D1	8	17
2	D1 + D2	7	11
3	D1 + D3	13	14
4	D1 + D4	7	14
5	D1 + D5	15	27
6	D1 + D6	9	10
7	D1 + D7	11	14
8	D1 + D8	10	5
9	D2	10	12
10	D2 + D3	10	5
11	D2 + D4	3	16
12	D2 + D5	2	4
13	D2 + D6	10	11
14	D2 + D7	14	4
15	D2 + D8	11	16
16	D3	12	5
17	D3 + D4	11	9
18	D3 + D5	20	8
19	D3 + D6	16	20
20	D3 + D7	12	3
21	D3 + D8	9	18
22	D4	16	9
23	D4 + D5	8	10
24	D4 + D6	9	15
25	D4 + D7	13	18
26	D4 + D8	8	11
27	D5	8	10
28	D5 + D6	15	26
29	D5 + D7	8	8
30	D5 + D8	9	11
31	D6	5	15
32	D6 + D7	14	8
33	D6 + D8	13	6
34	D7	2	25
35	D7 + D8	4	7
36	D8	5	19
37	11	18	17
38	176	14	11
39	192	12	9

In connection with the above experiment it is worth recording that culture No. 11 was isolated in 1920. The fungus had, therefore, retained its virulence during 11 years of artificial culture.

Although the results of this experiment seemed perfectly conclusive, they were open to possible criticism, since the experiment was performed

out of doors. A second experiment, therefore, was planned, using young potted trees grown in the greenhouse and known to be free from curl. The performance of this experiment was delayed by lack of funds for the necessary equipment. Finally, in the spring of 1934, several such trees were inoculated with single-ascus cultures and others with monosporous cultures. These trees were then placed under a spray of water from a Tyler vapor head and kept there for several days. Curl did not result.

It was during the progress of this experiment that the paper by Fitzpatrick (8) was received in which successful inoculation of peach trees with monosporous cultures of the fungus is reported. Apparently, Fitzpatrick's arrangements to secure infection were better than those of the writer. All of the observations and experiments here reported were completed in 1931, and publication has been delayed only for this final confirmatory experiment. Under the circumstances, it does not seem worth while to repeat it.

Since infection resulting in typical curl can be effected by conidia descended from a single ascospore, it is clear that in *Taphrina deformans* copulation of conidia is not a necessary antecedent to infection, nor essential to the life history. However, in the case of penetration shown in figure 1, E, copulation had apparently preceded emergence of the germ tube, and this tube bore two pairs of nuclei. This observation together with that of imperfect cell unions and of joined tubes from different conidia reported above indicate that copulation does occasionally occur. This phenomenon may, perhaps, be reminiscent of a once functional sexual union, such as seems to occur regularly in *Taphrina epiphylla.* There is, of course, no direct evidence that copulation is a necessary antecedent to infection in the case of *T. epiphylla.* Further, Fitzpatrick (8) is in error when he states that the writer has postulated that copulation must precede the formation of a binucleate germ tube in the case of *T. deformans.* Clearly, the dicaryophase in this fungus is normally initiated by division of a nucleus of a single conidium, as stated by Eftimiu (7), and as shown by the observations on penetration made by both Fitzpatrick and the writer.

Nucleation of Cells Formed in Culture

The bud conidia formed in culture are usually uninucleate, and a new bud receives one daughter nucleus (Fig. 5, A, B, C). Short straight tubes put forth by conidia are usually uninucleate (Fig. 5, D). The long-twisted, tubes which sometimes join at their tips, are often binucleate or may show several pairs of nuclei (Fig. 5, E, F, G, K). In such cases the nucleus near the tip of a tube is more prominent and stains more brightly (Fig. 5, G, K). Occasionally, slender hyphae with binucleate, thin-walled cells are observed, as well as binucleate conidia (Fig. 5, L, H, I). In old cultures larger hyphae with binucleate, thick-walled or thin-walled cells are found (Fig. 5, N). Conjugate division of the nuclei of binucleate cells

has been observed, but the number of chromosomes could not be determined (Fig. 5, M). Rarely, plate septa have been seen between cells of thick-walled hyphae, and such cells have been seen to separate by dissolution of this septum. Fitzpatrick (8) states that the nuclei of tubes observed by him in culture are spaced far apart and do not suggest a true dicaryon. A dicaryon does occur, according to this author, in an infecting hypha. This seems a rather profound effect of the host upon a tube emerging from a conidium.

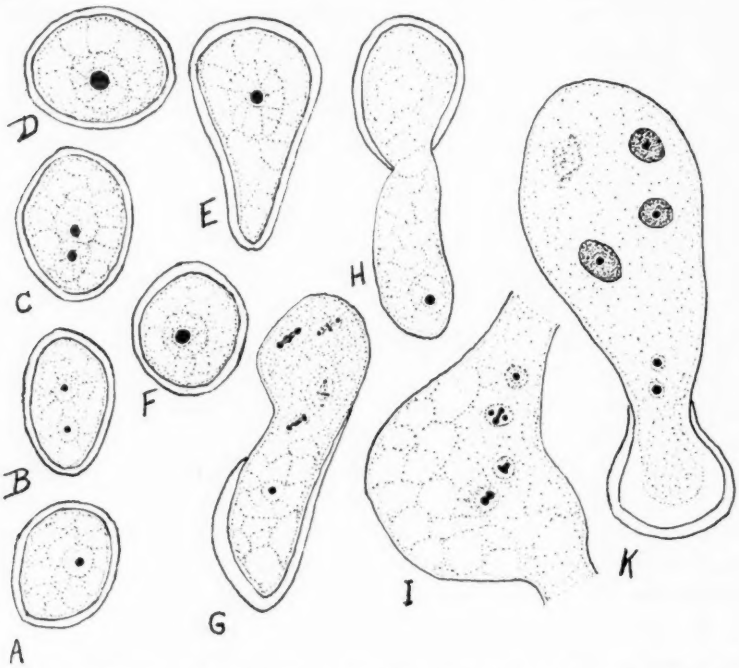

FIG. 6. Ascogenous cells and asci formed by *Taphrina deformans* in culture. A. Uninucleate; B. Binucleate ascogenous cells. C. Nuclear fusion in an ascogenous cell. D, E. Ascogenous cells with fusion nuclei. F. Ascogenous cell from hymenium on leaf to show comparative size. H. Young uninucleate ascus. G. Ascus with 5 nuclei, 4 of which are dividing. I. Portion of abnormally shaped ascus in which the nuclei contain brightly staining bodies, suggesting nuclear division in which the spindle fibers are not evident. K. Ascus with 3 spores, a degenerating nucleus, and two additional well-defined nuclei.

Thick-walled resting cells, previously described (14), may be uninucleate or binucleate (Fig. 6, A, B). Stages of nuclear fusion have been observed (Fig. 6, C, D) and cells with large, single, brightly staining nuclei that seem to be fusion nuclei (Fig. 6, D, E). The size and shape of these resting cells is variable, but often they resemble closely ascogenous cells found on the leaf (Fig. 6, F). It is believed that they are, in fact, ascogenous cells. Their mode of germination is additional evidence for this belief, and that

they have been observed to arise saprophytically in nature (14) is not evidence against it.

Resting cells germinate by rupture of the wall and the extrusion of a rather broad protoplast that may show contortions and twisting like that observed in tubes arising from conidia; in fact, it may occasionally be so narrow as to greatly resemble the latter. Some of these emergences are certainly asci. They bear a single prominent brightly staining nucleus (Fig. 6, H). The resting cell itself may at this stage show a degenerating nucleus, or the fusion nucleus may migrate to the young ascus. This appears to agree with the observation of Ikeno (9) who states that stalk cells are apparently cut off at different times, and that sometimes a nucleus ("chromatin bodies") remains in the stalk cell. Division figures of the fusion nucleus have been observed but the chromosomes could not be counted, nor could any synapsis stage be clearly seen, so that it cannot be definitely asserted that this is a reduction division. Asci with several nuclei, and with some of them in division have been observed (Fig. 6, G, I). One has been seen with 8 nuclei so well defined as to suggest that spore formation might be about to occur. Others have been found with 2 and 3 fully formed spores (Fig. 6, K). These asci formed in culture show some abnormality of shape, but they are comparable in size to those found in nature.

The significant part of these observations is that cells belonging to the dicaryophase and diplophase may develop in culture. It is further of interest that cells of all the types described above have been observed in monosporous, as well as in single-ascus, cultures.

Ascogenous cells and asci are found only in comparatively old cultures. While they have been formed on a variety of media, they occur more commonly in cultures on certain vegetable plugs, such as beet, carrot, and sweet potato.

ORIGIN OF THE SAPROPHYTIC PHASE OF THE LIFE HISTORY

All of the cultures of *Taphrina deformans* described by the writer (15), Klebahn (11), and Wieben (30) have been initiated by ascospores. An apparent exception occurred in the case of cultures isolated by the writer (15) by planting surface-sterilized pieces of curled leaves in tubes of potato-dextrose agar. It was at first believed that mycelium grew out from these pieces on to the agar. In continuing these isolations small pieces of leaf, approximately $\frac{1}{2} \times 1$ mm. were compared with larger pieces, 3 mm. $\times$ 5 mm. The small pieces soon turned dark brown and were evidently dead. No colonies developed from them. Most of the large pieces remained bright colored and apparently alive. By occasional transfer to fresh tubes of agar some of them were kept alive for twelve months. Around some of these pieces, colonies of *Taphrina deformans* developed from time to time.

In several cases the colony did not originate in contact with the leaf fragment but on the agar a few millimeters away. Microscopic examination of several tissue-fragments showed that the mycelium had remained alive in them and that asci and spores had formed on their surfaces. Successful cultures had apparently arisen from ascospores ejected onto the agar. It must be concluded that isolation from the interior of leaf tissues cannot be accomplished, and that cultures must be initiated by ascospores or conidia.

In nature *Taphrina deformans* is strictly parasitic in its diploid phase and saprophytic in its haploid phase. Saprophytic growth must be initiated by haploid cells, ascospores or conidia, but cells of the diploid phase may develop in culture.

SUMMARY

A review of the literature shows no convincing evidence that *Taphrina deformans* possesses a perennial mycelium. There is, however, evidence that conidia derived from ascospores by budding may survive and cause infection the following spring.

Attempts to isolate *Taphrina deformans* from the surfaces of healthy peach twigs were, as in the writer's earlier work, unsuccesful.

Inoculation experiments have shown not only that conidia may infect the young unfolding leaves, but that they may do so after surviving on twig surfaces for more than a year. A bagging experiment has shown that, when ascospores are prevented from reaching twig surfaces, curl does not occur the following spring.

An experiment in summer spraying, planned to give evidence as to the survival of conidia on twig surfaces, was inconclusive. Field observations on peach leaf curl have shown that conidia may survive for more than one season and still be capable of infecting, and that late infections on well-expanded leaves may occur. There may even be a small amount of secondary infection in a season when early curl occurs.

Hibernation of conidia probably is not confined to the surface of bud scales, as stated by some writers. Conidia may be believed to survive on all surfaces of the dormant tree.

A study of the occurrence and distribution of mycelium in diseased peach twigs has shown that perennial mycelium does not exist.

Infecting conidia send germ tubes through the outer wall of the lower epidermis (perhaps, in the case of late infections, through the upper epidermis, as well). Some lateral growth may occur beneath the cuticle. A hypha then grows in the radial wall between two adjacent epidermal cells to establish the subepidermal intercellular mycelium. After some occupation of the interior intercellular-space system of the leaf, the mycelium forms a second subepidermal layer next to the upper epidermis. From this mycelium branches grow up in radial epidermal walls to give rise to

the subcuticular hymenium. Asci occasionally are borne on the lower, as well as the upper, surface of the leaf.

Evidence has been obtained from a study of cells in culture and of stained preparations of occasional copulation of conidia. Some evidence suggests that copulation between long irregular tubes from conidia may sometimes occur. A case of apparent copulation preceding penetration has been observed. Careful study of conidia from monosporous cultures has, however, shown no evidence of copulation between genetically different conidia. Wieben's observations as to copulation of conidia of *Taphrina epiphylla* and *T. klebahni* have been repeated and confirmed. The most that can be believed regarding copulation of conidia of *T. deformans* is that it is occasional and is perhaps reminiscent of a once functional process. The dicaryophase, in this fungus, normally originates by division of the nucleus of one conidium.

An inoculation experiment has shown that conidia descended from each of the eight ascospores of an ascus can infect. Copulation of conidia is therefore not a necessary antecedent to infection.

Incidental to this experiment was the fact that conidia may cause infection after being grown in culture for 11 years.

Cells of the dicaryophase and diplophase may be formed in culture. Such cells are: binucleate conidia, hyphae with binucleate cells (showing conjugate division of nuclei), ascogenous cells, and asci. Asci formed in culture are imperfect and often abortive, but a few have been found containing ascospores.

Although diploid cells may develop in culture, saprophytic growth (as evidenced by growth in culture) must be initiated by haploid cells, ascospores, or conidia.

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EXPERIMENTS ON THE CONTROL OF THE ROOT-KNOT NEMATODE IN THE FIELD WITH CHLOROPICRIN AND OTHER CHEMICALS[1]

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Concurrent with greenhouse and small-plot experiments on the use of chloropicrin and other chemicals for nematode control reported in another paper (7), 2 comprehensive field tests were installed, in the spring of 1931. Both were in Hawaiian pineapple lands known to be very heavily infested with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Both were installed according to the Latin Square plot arrangement in order to reduce to the minimum the influence of soil heterogeneity in infestation. Undoubted value of chloropicrin for nematode control and for improvement of crop yields in nematode infested fields is indicated. Complete accounts of the experiments are given in this paper.

LITERATURE REVIEW

No attempt is made to review here all the extensive experimental work with chemicals for nematode control. As chloropicrin is of major interest, special literature studies were made on that chemical. It has for several years received the attention of entomologists, particularly as a fumigant for stored-product insects, but to some extent, also, for soil treatment. Roark (11) and Gersdorff (5) have supplied very complete bibliographies on all phases of the work with chloropicrin up to October, 1930. The work of Matthews (10) is significant of the possibilities with this particular chemical for nematode control, though her plot experiments with tomatoes were not highly promising in the matter of yield results. Her pot studies show striking benefits from the use of the chemical. Johnson and Godfrey (8), in row-treatment experiments, show striking results from chloropicrin applications, in reduced nematode population of the soil and improved pineapple yield. Godfrey, Oliveira, and Hoshino (7) report from 99 to 100 per cent reduction of nematodes where the chemical is adequately confined in the soil by gas-tight covers. Russell (12) reports good results in nematode control, and attributes remarkable root stimulation to the partial sterilization of the soil. He gives a number of illustrations showing this effect. Stone and Campbell (15) obtained good results with chloropicrin in controlling wireworms. Their use of it was as a contact applica-

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tion, in the form of an emulsion, rather than as a fumigant. Korab and Bootovsky (9) have recently reported tests with chloropicrin against the sugar-beet nematode, *Heterodera schachtii* Schmidt, with applications of 200, 400, and 800 liters per hectare. Increases in yield up to 500 per cent and of 2 per cent in sugar content were obtained. Four hundred liters per hectare (586 pounds per acre) is the ultimate rate of application recommended by them.

Several of the papers mentioned by Roark and Gersdorff (11, 5) and certain more recent papers indicate that chloropicrin has value as a soil fungicide and bactericide. Cooke (2) attributes apparent stimulatory effect of chloropicrin on sugar cane in Hawaii to its control of injurious soil fungi, particularly Pythium species, that attack certain varieties; Shchepetilnikova (13) reports it as an effective remedy against the fungi associated with flax sickness, at the same time augmenting the beneficial bacterial flora. Some of the improved plant growth resulting from experiments on the control of injurious soil fauna, therefore, may result in part from its effects on soil flora.

EXPERIMENTAL METHODS

The methods of preparation of the experimental areas, injection of chloropicrin, and subsequent procedure, were similar in the two experiments. The fields were plowed to the usual depth of about 12 inches (30 centimeters) and otherwise prepared for planting according to regular plantation practice. The specific areas for the experiments were then measured and the plots staked and labelled in accordance with detailed plans. The chemicals were injected into the soil in measured dosages, evenly spaced in the plant bed, by means of Vermorel carbon bisulphide injectors to depths of 6 to 8 inches. All beds were covered with the usual tar-impregnated pineapple mulching papers and the edges of the paper were covered to a depth of about 2 inches with soil. Differences in details of application between the 2 experiments are explained elsewhere. At safe periods after the treatments (not less than 48 hours) pineapple slips were planted through the papers originally used as covers, following regular field practice. No injury to the plants from the chemicals was evident at any time.

Measurements of the effectiveness of the treatments were then made in the following several different ways:

(1) Counts of numbers of Heterodera galls on the roots of cowpea indicator plants, planted in the pineapple plant beds for the purpose during the first month after treatment, after the method described in another paper (6).

(2) Thoroughgoing studies on random-selected pineapple plants removed with root system intact at 3 to 4 months after planting.

(3) Occasional photographs showing plant-growth differences between plots.

(4) Yield records at "plant crop" or first crop, harvested the second summer, from 15 to 18 months after planting.

The results throughout are analyzed statistically, according to the Latin Square system explained by Fisher (4) and Student (17), and significances of differences are determined by Student's tables (16) and Sheppard's tables (14).

Experiment 1.—A Latin Square Soil Fumigation Experiment with Chloropicrin at Different Rates of Application.

This experiment was installed in a Libby, McNeil, and Libby field in the Pupukea section, on the north-west slope of the Island of Oahu. The selected portion of the field had already been planted to pineapples the previous fall, but in February, 1931, it showed an extremely high percentage of failure and all plants examined showed heavy development of nematode root galls. Rather than replant irregularly, the plantation manager had the entire failing block of plants removed with a view to replanting throughout. The area was allotted to this experiment.

The planting plan in this section was the 4-row system, that is, 4 plant rows in the bed; the plants to be staggered, 15 inches apart in the row; plant beds covered with 54-inch-wide mulching paper; the beds spaced at 8-foot intervals, center to center, making about 17,800 plants to the acre. The area to be devoted to this experiment was about 40,000 square feet, or just short of 1 acre. This provided space for 36 plots of 5 beds each, with rows 27 feet long. These were arranged according to the Latin Square plan of random selection, as follows:

6	5	4	3	2	1	A plots, control, no treatment.
						B plots, control, no treatment.
F	B	E	A	C	D	C plots, chloropicrin, 130 pounds per acre.
A	D	F	C	E	B	D plots, chloropicrin 160 pounds per acre,
C	F	B	D	A	E	with extra cover (see text).
E	A	D	B	F	C	E plots, chloropicrin 160 pounds per acre.
B	E	C	F	D	A	F plots, chloropicrin 97 pounds per acre.
D	C	A	E	B	F	Each plot, 1,080 square feet.
						Total area, 39,000 square feet.

Certain irregularities in the shape of the field made for lack of complete perfection in the Latin Square arrangement, but these did not interfere with the legitimacy of the Latin Square system of analysis of results, and this paper is not complicated by the detailed explanation. Plot F in column 6 was smaller than the rest. This irregularity is provided for in the analysis of results.

Applications of treatments commenced on February 24. The applicators were adjusted to make the correct delivery per injection. It was

necessary, for some plots, to dilute the chloropicrin slightly with denatured alcohol or gasoline, so that each injection would deliver exactly the right quantity of chloropicrin. The basic rate of application, that used in F plots, was 2 cc. per hole in 3 lines of holes in each bed, spaced at 15-inch intervals, a total of 65 holes per bed, making 130 cubic centimeters per bed or 650 cubic centimeters per plot. On this basis the rate of application was 97 pounds per acre (approximately 54 liters, or 90 kilograms per hectare). Plots D and E, receiving the heaviest applications, received exactly the same amount per injection but with 2 additional lines of holes along the edge of the mulching paper. This gave a rate of application 2/3 greater than the basic rate, or approximately 160 pounds per acre (150 kilograms per hectare). The C plots received 1/3 more chloropicrin per injection spaced as in F plots, making about 130 pounds per acre (120 kilograms per hectare).

The action of the chemical upon the valves of the applicators made them somewhat erratic in their delivery, so that constant checks upon the quantities delivered had to be made. These quantities varied sometimes in different plots of the same treatment, usually within 5 or 10 per cent of the desired, but as high as 25 per cent off in 1 or 2 beds. Unfortunate as this was in its tendency to bring about irregularities in results without opportunity for making due allowance in calculations, it was unavoidable with the equipment available. Detailed notes made for each plot show actual applications to be slightly higher on the average than the desired quantity. It is certain, however, that the relative relationships of quantities used in plots C, D, E, and F were very close to the ratios planned.

Since, through misunderstanding, the mulching paper was already laid by the plantation men in preparation for regular planting at the time planned for application of the chemical, it was necessary to insert the chloropicrin into the soil through holes punched through the paper with the applicators. A second man with each applicator covered the holes with earth, and the soil at each point was firmly compacted with the foot. This prevented quick escape of the gas through the hole, but did not permit of the degree of retention that might have been attained had unbroken covers been used. Correct 15-inch spacing of points of application was attained by the use of marked planting lines, the same ones that were used later for planting the pineapples in the same beds. Extra covers, extending from near the centers of beds to about 6 inches from the centers of "middles" between beds, were placed over D plots. This was designed to bring about better retention of the gas in the soil. The extra covers were removed before pineapples were planted. The operation of installation was delayed by a period of intermittent showers until March 5, when final beds were treated. At no time were applications made in soil that was nearly satu-

rated with moisture, but only in soil of loose loamy texture. Pineapples were planted within a few days after treatment. A line of plants was omitted between plots in the "columns" (see planting plan) to permit of sharp plot demarcation. Figure 1 is an aerial view of the experimental area taken about 11 months after planting.

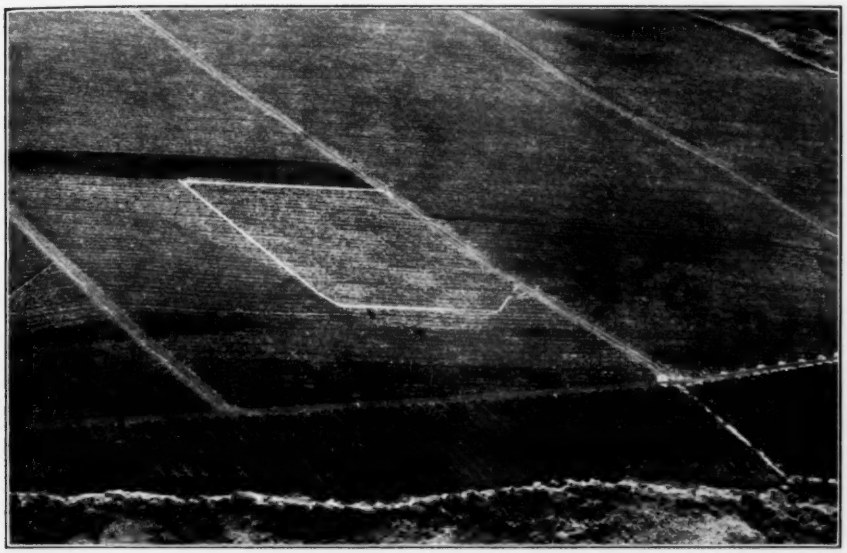

FIG. 1. Airplane view of the area used for experiment 1, marked with fine white lines. The road to the right of the area corresponds to the base line of the layout plan shown on page 78. The plots are in the shape of parallelograms rather than rectangles. In the original photograph there is evident a faint, irregular, checker-board effect, brought about by the contrast between treated and control plots, which can be identified by referring to the before-mentioned layout. The dark line in the lower left corner of the experimental area is the shadow of the airplane from which the picture was taken. Photographed by the 11th Photo Section, Air Corps, Luke Field, T. H., March 10, 1932, about 11 months after the pineapples were planted.

RESULTS FROM EXPERIMENT 1

Within 3 months after planting distinct differences became evident in vigor of plant growth, between treated and nontreated plots. At from 3 to 5 months the relative under-size in nontreated plots was accompanied by chlorosis, a distinct yellowing, as compared with the treated plots. Evidently, reduced root development in nontreated areas made for a reduction in iron absorption. Sprayings with iron sulphate according to regular plantation practice so overcame this difference that, after 6 months, color differences were less pronounced. Airplane pictures, taken during the first year of growth, at 5 and 11 months after planting, through the courtesy of the 11th Photo Section, Air Corps, Luke Field, T. H., show fairly well

such plot differentiation. Figure 1 is a reproduction of one of the later photographs. Plot differences in size of plants increased until near the time of fruit production and striking differences persisted throughout the life of the plants, similar to those shown by Johnson and Godfrey (8) in their row-treatment experiments. Figure 2 shows the adjacent lines of plot 1–D, right, and nontreated control, left, with size differences strikingly evident, at 12 months after planting. Certain weeds, particularly the "popolo" (*Solanum nodiflorum* Von Jacquin), growing in the pineapple rows, likewise showed striking differences in growth. When pulled, such weeds showed outstanding differences in amount of root-gall development, but no quantitative records were made by which such differences can be presented. Quantitative measurements of the effects of treatments in this experiment consisted of root-gall count on indicator plants, grown at the

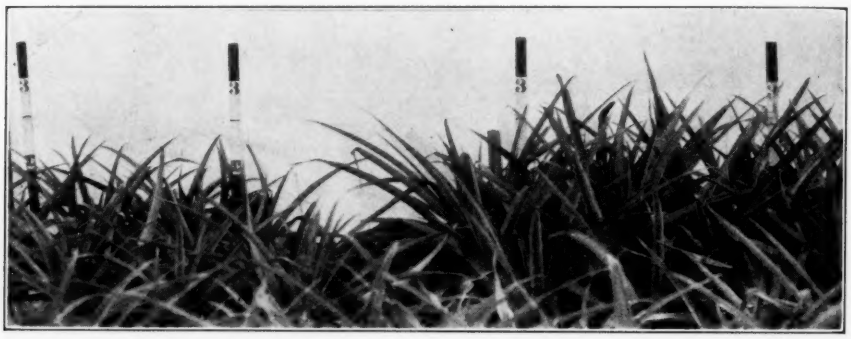

FIG. 2. Photograph, taken about a year after planting, showing the striking difference in size of pineapple plants between a nontreated area, left, and a chloropicrin-treated area (1–D) right. The marked stakes are flush with the ground level at the zero point. Treated plants are over 3 feet high, while nontreated are barely over 2 feet.

edges of the paper and in holes cut through the paper with hoes, and of yield records.

Results in Root-gall Counts on Indicator Plants.—In order to obtain an earlier quantitative estimate of the effects of the treatments than could be obtained from observations on pineapple plants, Whippoorwill cowpeas were planted shortly after pineapple planting, in holes cut through the mulching paper, in the middle 3 beds of every plot. Moisture conditions were favorable for sprouting, and plant growth was good. The plants were removed, after about 1 month of growth, with root systems as complete as was possible, by first loosening the plant with a trowel and pulling gently on the top. Since entire root systems were not obtained, considerable irregularity in root volume removed was unavoidable. It is obvious, therefore, that no high degree of accuracy in nematode-population estimates could be expected. Where great differences occurred because of

treatments, such differences might be expected to appear in gall-count readings. Smaller differences, such as those between treatments, might not be expected to be indicated so satisfactorily by this rough method. Actual numbers of plants per plot examined ranged mostly between 300 and 600.

For purposes of comparisons between plots the direct readings in terms of root-gall average counts, by plots, was considered satisfactory without multiplication by a constant factor, as suggested elsewhere (6), for transforming the results to an approximation to actual soil infestation. In order to eliminate large decimals in the analysis of results, readings are analyzed in terms of numbers of galls per 100 plants. Without complicating this paper by the details of preliminary calculations, the analysis of readings by Fisher's Latin Square method resulted in a table of variances, as follows:

TABLE 1.—*Table of variance in root-gall count per 100 nematode-indicator cowpea plants*

Variance due to	Degrees of freedom	Sums of squares	Variance	Standard deviation
Treatments	5	10383759.18		
Columns	5	1318579.53		
Rows	5	1308133.78		
Remainder	20	6144866.13	307243.30	554.3
Total	35	19155338.62		

As is to be expected from the rough method of measurement, the standard deviation is very high, so high, in fact, as to appear unfair to the more even distributions of the better treatments. (The standard deviation of the distribution of readings by the normal curve method, on D plots, for example, is only 66.3, while that on E plots is 102.5.) Continuing with the Latin Square analysis, however, 554.3 is multiplied by the factor $\frac{2\times 21}{6}$ to obtain 1466.6 with which plot differences are divided to obtain "Z," used in Student's tables.

The differences between plots in gall counts, and percentages, and the significances of differences are shown in table 2. The average gall count in A and B check plots together is 1240.4. The P values are tabulated as the criterion of significance. Since a P value of .997 is equivalent to a $\frac{D}{PE}$ of 4, P values of table 2 of .997 or higher are thoroughly acceptable assurance that differences are due to treatments and not to chance, while a P value only slightly less is still very highly significant. It is to be seen that, even with the rough measurement used (gall count on roughly removed plants) with very high standard deviation, reductions in nematode

TABLE 2.—*Comparisons of plot treatments in root-gall counts per 100 nematode-indicator cowpea plants, with significances of differences*

Plot	Treatment (chloropicrin, lbs.)	Gall count	Comparison with	Difference	Percentage difference	P	Significance (odds)
$\frac{A+B}{2}$	0	1240.4	.....	.....	.....	.....	.....
C	130	67.5	Checks	1172.8	94.5	.9993	1428 to 1
D	160	69.0	"	1170.5	94.4	.9987	768 to 1
E	160	69.3	"	1171.2	94.4	.9988	832 to 1
F	97	433.9	"	806.5	65.0	.9862	72 to 1
E	160	69.3	F	364.6	84.0	.8489	16.6 to 1

count of better than 94 per cent occur with the 3 higher treatments, with certainty of significance, while with only 97 pounds of chloropicrin per acre 65 per cent reduction occurs with odds 72 to 1 in favor of significance. The rough measurement did not serve to show any significant difference between 130 and 160 pounds per acre, and the difference between the 97- and the 160 pound treatments, while a large one, is not highly significant statistically. The measurement of differences in plot yields, made with much greater accuracy, shows plot differences of higher significances.

Results in Differences in Total Plot Yields.—Beginning in June, 1932, and continuing into September, during the normal ripening period of the pineapple crop, yield records were taken on all plots. Accumulated totals of numbers of plants harvested and of yields were taken without effort to obtain individual plant records. Unfortunately, one error was made when, through misunderstanding, the regular plantation-field harvesting crew began to pick fruits and removed a portion of those from plots B and D in column 6. This removed the possibility of complete accuracy in total yield figures for these plots. In order to obtain a figure for purposes of calculation, the same values are allotted to these plots as was calculated accurately for the average of the other 5 plots of the same treatments. These plots were not outstandingly different from the average, and in all probability this figure is not far from the correct one. The same sort of adjustment

TABLE 3.—*Table of variances in total plot yields, in pounds of pineapples*

Variance due to	Degrees of freedom	Sums of squares	Variance	Standard deviation
Treatments	5	649032		
Columns	5	122609		
Rows	5	57293		
Remainder	20	120328	6016.4	77.56
Total	35	949262		

was made for plot F in column 6, since, as stated in the introduction to the experiment, this plot was considerably smaller than the others.

Table 3 shows the yield-data variances from which the standard deviation due to random error is calculated.

Multiplying 77.56, the standard deviation due to random error, by the factor $\frac{2 \times 21}{6}$, the constant 189.94 is obtained, with which plot differences are divided to obtain Z, used in Student's tables. The differences between plots in yields per plot and the percentage differences, together with significances, are shown in table 4. The average yield per plot of A and B plots together, taken as before for purposes of comparison, is 1022 pounds.

TABLE 4.—*Comparisons of plot treatments in yield per plot, in pounds of pineapples*

Plot	Treatment. Chloropicrin (pounds)	Yield pounds	Comparison with	Difference	Percentage increase	P	Significance (odds)
Checks	.....	1022	.....	.....	.....	.....	.....
C	130	1286	Checks	264	25.8	.9999	10000 to 1
D[a]	160	1327	"	305	30.0	.9999	"
E	160	1343	"	321	31.4	.9999	"
F	97	1164	"	142	13.9	.9980	500 to 1
E	160	1343	C	57	4.4	.9026	9.3 to 1
E	160	1343	F	179	15.4	.9997	3332 to 1
C	130	1286	F	122	10.5	.9953	212 to 1

[a] With extra cover for better confinement of the gas. See text.

All treatments, including even the very low one of only 97 pounds of chloropicrin per acre, showed undoubted increase in yields, the maximum being better than 31 per cent with one of the 160 pounds per acre applications. No difference between results from the 2 methods of application of D and E plots is evident, so the additional cost of an extra cover is not indicated. The 160-pound application was not significantly higher than the 130 pound rate, in results. Both the 160- and the 130-pound applications, however, were definite improvements over the 97-pound treatment. These results are subject, of course, to the slight error introduced by the uncertainty as to exact total yields in B and D plots in 1 series.

Still another analysis of yield results was made, on the basis of average weight per fruit. This figure was obtained as a result, by plots, of total yield actually harvested divided by total number of fruits harvested. The results are shown in table 5.

A complete Latin Square analysis of results, on the basis of even numbers of plants, was made. The standard deviation due to random error, as

TABLE 5.—*Average pineapple fruit weights, by treatments*

Plot	Treatment	Average number of fruits	Average weight per fruit (pounds)		Critical difference, 0.188
A	Control	342	2.99	3.01	
B	"	326	3.03		
C	130	345	3.74		
D	160	338	3.86		
E	160	356	3.74		
F	97	296	3.51		

calculated, is .103. The constant, for the determination of Z is .2725. For the sake of simplicity in making comparisons, the "critical difference" was determined, at or above which significance is completely reliable, with odds of 332 to 1 or higher. As shown in the table, this figure is 0.188 pounds. It is thus to be seen at a glance that all the treatments gave significantly higher yields per plant than the average of the checks. Likewise, C, D, and E show significantly higher averages than F. Other differences are not significant.

Experiment 2.—A Latin Square Soil Fumigation Experiment with Chloropicrin and Carbon Bisulphide.

This experiment was installed shortly after experiment 1 in a field in the heart of the best pineapple area in the interior of the island of Oahu and known to be very heavily infested with nematodes. The area selected, about 1 acre, had been planted to an experimental block of *Sesbania macrocarpa* Mühl., a highly susceptible legume, and it is certain that heavy reproduction of nematodes had occurred. This had been plowed under within the previous few months, and the field prepared for pineapple planting, according to regular practice.

The layout and installation of the experiment were similar to those of experiment 1, with differences as follows: The system of planting was a 2-row system, 2 plants in a cluster, with 20-inch spacing, beds 6 feet center to center, making approximately 17,000 plants per acre. Each plot consisted of 6 beds each 25 feet long, a total of 900 square feet or approximately 1/50 of an acre—a convenient figure for calculating quantities of chemicals needed per plot and bed. Each bed contained 30 clusters of plants.

The chemicals were applied with the Vermorel injectors used in experiment 1, in 2 lines of holes with 10-inch spacing, or just half that of the

pineapples, thus making for good general diffusion of the gases through the soil. Plots D and F of the chloropicrin treatments (see layout of experiment, to follow) received an extra line of treatments at either edge of the permanent mulching paper, directly opposite the plant clusters. Carbon bisulphide plots, receiving 900 cc. per plant bed, received 3 injections of 5 cc. each, in 60 different spots. Thus each planting point was well surrounded with injections. The applicators were adjusted for correct delivery, but unavoidable discrepancies, mostly in the direction of slight excess occurred. These are disregarded in calculations, throughout, inasmuch as relative proportions are very close to the desired.

The applications were made directly in the ground and the injection hole pressed with the foot to prevent too rapid immediate evaporation. Immediately after the completion of each bed of a block, mulching paper was unrolled to cover the bed and the edges were covered with soil. This gave better confinement of the gas than was obtained in experiment 1, in which the holes punched through the paper permitted of more rapid escape. In accordance with previous experience, recorded elsewhere (7), a higher degree of efficiency might be expected from this better confinement. In plots F and G (see layout) 2 extra strips of paper were laid on each bed extending approximately to the middles (between beds) for the purpose of still further contributing to the efficiency of gas confinement.

There were 7 treatments, including 2 controls, each replicated 7 times. The plots were arranged according to the Latin Square plan of random selection, as follows:

7	6	5	4	3	2	1	Each plot consists of 6 beds.
D	C	B	E	G	F	A	Beds 25 feet long, 6 feet center to center.
B	E	G	C	D	A	F	Area of each plot, 900 square feet.
F	D	C	A	E	B	G	60 plants to each bed, 360 to the plot.
G	A	D	F	C	E	B	Total area, 44,100 square feet.
E	F	A	G	B	C	D	
C	B	F	D	A	G	E	
A	G	E	B	F	D	C	

Treatments were as follows: A, control, nontreated, except for usual mulching paper cover; B, chloropicrin 150 pounds per acre; C, carbon bisulphide, 750 pounds per acre; D, chloropicrin, 170 pounds per acre; E, control, identical with A; F, chloropicrin 170 pounds, with extra cover; G, carbon bisulphide 750 pounds, with extra cover. The position and surroundings of the experimental area are shown in figure 3, an airplane view, taken from approximately 700 feet about 5 months after planting.

Shortly after the treatments, pineapple slips were planted through the mulching paper according to regular plantation practice. Thereafter, in general, regular field cultivations along the rows were avoided in order to

keep to a minimum the spread of infestation from one plot to another. Unfortunately, through misunderstanding as to responsibility, such cultivation occurred at least once. Weeds were removed by hand or by hoes. Volunteer Sesbania plants developed in considerable abundance throughout the experimental area and were treated as weeds. Occasional observations on such plants disclosed undoubted effectiveness of treatments in reducing soil infestation, for those in the control areas were as a rule very heavily infested, while those in treated plots were frequently quite free from galls.

FIG. 3. Airplane view of the area used for experiment 2, 5 months after planting, 3 of the corners being marked with white strips of cloth. The road on the right of the area is the head line corresponding to that of the layout shown on page 71, column 1 being on the nearer side of the picture. Contrasts between nontreated controls and the better treatments are clearly distinguishable in the original photograph. Vacant spaces left by the removal of plants for detailed measurements, are clearly shown. The photograph was made possible through the courtesy of the 11th Photo Section, Air Corps, Luke Field, T. H.

No quantitative studies were recorded on such plants, however. Particular care was taken throughout the growth of the pineapples, up to plant-crop stage of development, to prevent, by spraying with oil emulsion as frequently as necessary, the development of mealy-bug infestations, whose effects in producing "mealy-bug wilt," as reported by Carter (1), would seriously vitiate the uniformity of nematode-control results.

As with experiment 1, within 3 months after planting striking differences between treatments became evident, in general vigor of plant growth,

as indicated by an unhealthy yellowish-green color, as well as by dwarfed growth. Figure 3 shows the checkerboard effect to some extent, but not nearly so strikingly as it actually appeared, because of lack of good color contrast in the photograph. Figure 4 shows comparative sizes of the adjacent rows of plots 5–E, left, and 4–B, right, on April 14, 1932, 12 months after planting. Reference to the layout plan and perhaps to figure 3 will determine exactly where the picture was taken.

Results of the treatments were measured by 3 separate studies, as follows:

(1) Counts of galls on cowpeas, planted as nematode indicator plants.

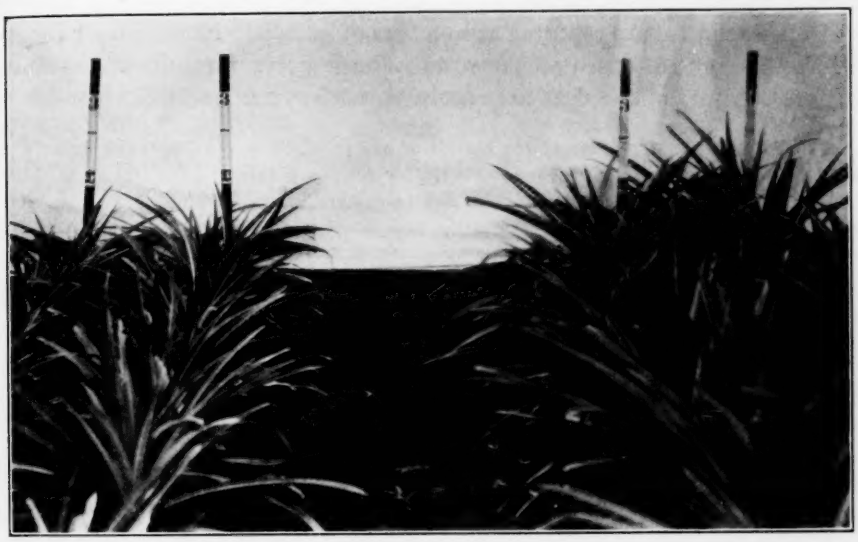

FIG. 4. Photograph to show size differences between nontreated and treated plots in experiment 2, at about a year after planting. Left, plot 5–E, control; right, 4–B, chloropicrin 150 pounds to the acre. Note that the treated plants are about a foot taller than the nontreated.

(2) Measurements and gall counts on roots, and weights of tops, of 16 pineapple plants per plot, removed from the middle 4 beds of every plot from 3 to 4 months after planting.

(3) Complete plant-by-plant yield records made at plant-crop harvest during the summer of 1932.

It had originally been planned to go still farther into the analysis of results with yield records and, immediately afterward, complete root studies on representative random-selected plants from every plot, for the purpose of calculating correlations between root condition and yield on the same plants. Circumstances, however, prevented the consummation of these plans.

Results in Root-gall Count on Indicator Plants.—Whippoorwill cowpeas were planted through holes in the mulching paper, between the pineapple rows and at the edge of the paper, shortly after the pineapple plants were set and before the ground became shaded by them. The stand was somewhat uneven, but always the numbers were sufficient to provide good plot readings. The plants were removed when about a month old, somewhat roughly, as in experiment 1, but permitting of gall-count classifications, by treatments, with highly significant differences. The numbers of plants upon which observations were made were somewhat variable, but, as a rule, there were well over 100 per plot. Results were reduced to average numbers of galls per plant for each plot, and, in order to eliminate large decimals, these figures are reported as numbers of galls per 100 indicator plants.

For the sake of space economy, only the important results of the Latin Square analyses of the data are recorded, as shown in table 6.

TABLE 6.—*Comparisons of the results of soil-fumigation treatments in experiment 2, in root-gall count per 100 nematode-indicator cowpea plants*

(*Standard deviation due to random error,* 1372
Critical difference, 2262)

Plot	Treatment pounds per A.	Gall count	Comparison with	Difference	Percentage reduction
Checks	.....	10741	.....	.....	.....
B	Chloropicrin 150	1810	Checks	8931	83.1
C	Carbon bisulphide 750	6445	"	4296	40.0
D	Chloropicrin 170	1297	"	9444	88.0
F[a]	Chloropicrin 170	831	"	9910	92.2
G[a]	Carbon bisulphide 750	4660	"	6081	56.6
G			C	4635	80.0
B			C	1785	27.7

[a] Extra covers were used over these plots; see text.

Even though the standard deviation due to random error is rather high, all differences shown—that is, all treatments compared with checks, and all chloropicrin treatments compared with carbon bisulphide—are highly significant. The final comparison shown in the table, carbon bisulphide with and without the extra cover, shows considerable difference, with the odds 65 to 1 that the difference is due to the difference in treatment rather than to chance. The differences between chloropicrin treatments are not significant. The additional cover used in F was not of any significant value. The effects of the reductions that were brought about by the treatments in nematode population, on gall count on the roots of the planting of pineapples, and on root and top development of that crop, are shown in the next section of the paper.

Results in Readings on Random-selected Pineapple Plants.—The plants for this study were selected by marking with stakes the 5th and 10th hills of 2 plants each in the middle 4 beds of every plot, half of them on the east and half on the west row of the bed. Thus 16 plants were taken from each plot, a total of 784 plants from the entire experimental area. Beginning in July, 1931, about 4 months after planting, plants were carefully excavated with root systems intact, a very painstaking operation, requiring over a month for completion. The order of removal was such as to distribute evenly, by treatments, the factor of the influence of time on growth; this factor, therefore, can be ignored. The plants were taken to the Honolulu laboratory, where root systems were washed and the following complete records were taken:

Total number of roots per plant, counting as such only the main large roots, arising directly on the planting piece; total length of root system per plant, obtained by adding the lengths of all main roots counted; total volume of root system per plant; total number of terminal nematode root galls per plant; total number of non-terminal nematode root galls per plant; total number, terminal and non-terminal galls together; total number of small galls on lateral rootlets; total number of prominent *Tylenchus brachyurus* lesions; total number of roots less than 6 inches in length; total number of roots 12 inches or more in length; total weight of plant, exclusive of roots.

The results, in averages only and by treatments, of these various studies are given in compact form in table 7. For the sake of ready visualization of the treatment differences, the plots are arranged in the table with the check-plot average first, followed by the carbon bisulphide treatments, and finally the chloropicrin treatments. In the table the various features measured are lettered for ready reference for correlation studies that appear later. Item "a" consists of the earlier derived data on indicator-plant, root-gall readings. Items "m" and "n" consist of the yield data brought forward from the next section of this paper for convenient comparison with the other data.

The rank, in order of efficiency of treatments, is obvious in most cases. With greatest efficiency one finds (a) the lowest root gall counts; the highest figures for length and volume of roots (c and d); the lowest counts in e, f, g, h, and i; the lowest count in numbers of short roots (j) and the highest in long roots (k); the highest weights (l); and naturally the highest in yield records, (m and n). For number of roots (b) alone there is an apparent inconsistency with other results, though with significantly lower numbers in treatments C, G, D, and F than in the checks, there would appear to be a definite proliferation of unnatural early root development in the plants that were heavily parasitized by nematodes, similar to the prolif-

TABLE 7.—*Plot averages of readings on various bases of comparisons on nematode-indicator cowpea plants and pineapple plants to show results of treatments in experiment 2. The last column gives, for some cases, the minimum plot difference that indicates unquestionable significance. Significant differences are indicated, as per footnote. The bold-face superior numbers, indicate the rank of the treatments in order of effectiveness*

Designation	Basis of comparison	Controls (average)	Treatments					Critical difference and significant[a] differences
			Carbon bisulphide		Chloropicrin			
			C	G	B	D	F	
a	Indicator-crop gall count	**6** 107.4	**5** 64.5	**4** 46.6	**3** 18.1	**2** 13.0	**1** 8.3	22.5 s-1, -2, -3
Young pineapple plants								
b	Number of roots	**1** 34.3 ± .43	**3** 31.2 ± .68	**5** 30.1 ± .71	**2** 32.6 ± 1.06	**4** 30.7 ± .69	**6** 28.9 ± .91	s-1 ex. B, -2
c	Length of roots	**6** 291.5	**5** 305.8	**4** 312.4	**2** 387.0	**1** 392.1	**3** 356.1	60.34 s-1, s-3 ex. F
d	Volume of roots, cc.	**6** 25.5 ± .71	**5** 25.5 ± .56	**4** 26.1 ± 1.08	**2** 30.8 ± .59	**1** 32.8 ± 1.01	**3** 30.2 ± 1.24	s-1
e	Number of terminal galls	**6** 12.8	**5** 7.7	**4** 6.2	**3** 5.3	**1** 3.1	**2** 3.2	3.16 s-1, -2, -3 ex. B
f	Nonterminal galls	**6** 2.7 ± .15	**5** 1.5 ± .20	**4** .9 ± .11	**1** .27 ± .03	**3** .46 ± .03	**2** .30 ± .11	s-1, -2
g	Total galls	**6** 15.6 ± .66	**5** 9.2 ± .29	**4** 7.1 ± .53	**3** 5.8 ± .64	**2** 3.9 ± .09	**1** 3.2 ± .28	s-1, -2, -3

TABLE 7.—(*Continued*)

Designation	Basis of comparison	Controls (average)	Treatments					Critical difference and significant[a] differences
			Carbon bisulphide		Chloropicrin			
			C	G	B	D	F	
h	Lateral rootlet galls	6 29.0	5 6.0	4 11.0	1 2.8	3 3.6	2 3.4	s-1, -2, -3
i	Tylenchus lesions	6 10.5 ± .33	5 10.3 ± .33	3 8.2 ± .53	4 9.9 ± .28	1 6.3 ± .39	2 6.8 ± .46	s-1, ex. B
j	Number, roots < 6 inches	6 13.9 ± .26	5 11.9 ± .48	4 10.3 ± .44	3 7.7 ± .64	1 5.9 ± .27	2 6.1 ± .34	s-1, -2 for G, -3
k	Number, roots > 12 inches	6 10.0 ± .34	4 11.0 ± .32	5 11.3 ± .72	1 15.1 ± .21	2 14.9 ± .44	3 14.4 ± .44	s-1, -3
l	Weight of plants grams	6 423.7 ± 9.79	5 466.3 ± 13.7	5 475.0 ± 20.0	3 549.5 ± 17.6	1 578.0 ± 16.9	2 566.8 ± 12.3	s-1, -3
Pineapple yield records								
m	Total yield per plot, lbs.	6 792	5 1009	4 1041	2 1206	1 1207	3 1183	79.48 s-1, -2, -3
n	Weight per fruit, lbs.	6 2.74 ± .008	5 3.35 ± .012	4 3.38 ± .008	3 3.91 ± .012	1 4.03 ± .012	2 3.94 ± .012	s-1, -2, -3

[a] The figures for critical differences are inserted only for the cases in which complete analyses were made by Fisher's Latin Square method. The significance or nonsignificance of any particular difference is readily determinable in such cases. Other bases of comparson were analyzed by an assistant by the normal curve method, and significances by that method are indicated in the last column as follows: s-1, chloropicrin with checks; s-2, carbon bisulphide with checks; s-3, chloropicrin with carbon bisulphide, C (s meaning unquestionable significance).

eration of plant parts often produced by other types of parasitism. As an immediate effect this is probably beneficial to the plant, but it cannot continue indefinitely, and, in the long run, probably prevents normal new root development when it is later needed. The differences are not great, however, and this basis of measurement, because of the present uncertainty as to influences, can be disregarded in the further consideration of the data.

In all categories of comparison, except that of b, then, the treatments group themselves into 3 classes as to efficiency, always in the same order: first, the 3 chloropicrin treatments, B, D, and F; second, the carbon bisulphide treatments; third, the controls. The differences by which such classes are brought about are, in general, highly significant, statistically. (Note the single partial exception, in category i, and lack of either the chloropicrin-carbon bisulphide, or the carbon bisulphide-check significance in some of the categories of minor influence value.) In the table such grouping is indicated by subdivision into 3 parts by heavy lines. Within the groups, differences are not, as a rule, statistically significant. It is worthy of note, however, that plot G shows superiority over C in all but one of the bases of comparison, indicating that for carbon bisulphide the better confinement of the gas brought about by the additional cover made itself felt in results obtained. Such benefit from the extra cover in the case of chloropicrin treatments is not evident.

Results in Plant-crop Yields.—The ultimate criterion upon which judgment as to the value of any treatment for pest control is based is its effect on crop yield and quality. Measurements on yield for this experiment covered only "plant-crop," that is, the first fruiting, from July to September, inclusive, 1932. The data in total plot yields, analyzed by the Latin Square method, gave the following results.

Standard deviation due to random error, 48.58 pounds; critical difference (as explained heretofore) 79.48 pounds.

Average yields by treatments are appended to table 7. These data show at a glance the significant differences. They include all treatments over the checks and, very definitely, the chloropicrin over the carbon bisulphide. Percentage increases in yields from the treatments were as follow:

C	over	checks,	27.4	per	cent
G	"	"	31.3	"	"
B	"	"	52.2	"	"
D	"	"	52.3	"	"
F	"	"	49.3	"	"
B	over	C,	19.5	"	"

The economic significance of such increases is considered in the "Discussion" section of this paper.

An additional analysis of yield data, based upon individual fruit weights throughout, gives the results in average weight per fruit by plots.

These data are analyzed by the normal-curve method, since the populations were well over 2000 per treatment. The results are included in table 7 as the last category of comparison (n). They show much the same order as do total plot yields, the only differences in order being between chloropicrin treatments with which, in total yields, differences are not significant. Differences in numbers of fruits weighed, not accounted for by methods of record taking, make for the slight discrepancies that occur. Again, marked benefit from the chloropicrin treatments is evident.

Consideration of the Correlations Between the Different Bases of Comparison.—Further critical consideration of the data presented in table 7 shows, for the 3 main classes of results (chloropicrin, carbon bisulphide, and control) practically perfect rank-order correlation throughout, except for basis b, which may be disregarded. An analysis of this situation leads to the following conclusions:

(1) The nematode populations left after the treatments, as measured by basis a, accounts for the orders of degrees of nematode infection in the pineapple plants, as given in bases e, f, g, and h.

(2) These infections, in turn, affect root growth as manifested by bases c, d, j, and k.

(3) The extent of root growth probably is an important factor in plant growth, as shown by basis l.

(4) All such factors influence yield, as shown by m and n.

Thoroughgoing statistical treatment of these various categories of data is not included in the present paper, for they are not essential to its main purpose, *i.e.*, to show the results of the treatments on the pineapple crop. One correlation, however, that between a and m (indicator crop readings and plot yields), is reported as of definite interest in connection with nematode-control work, in general. Calculations were made on individual plot data, the population, therefore, being 49 plots. The step intervals were 10 galls per plant for indicator-crop data and 50 pounds per plot for yields. The result of this calculation was $r_{ma} = -.852 \pm .0265$; a surprisingly high correlation, considering the admittedly rough method of obtaining the data on gall counts. An undoubted influence of nematode population on pineapple yield is manifest, unless other factors, simultaneously influencing yields, were affected by the treatments, and nematode readings served as a measure of the effects on these other factors, as well.

The regression equation based upon this correlation is $M = -39.5\,A + 1242 \pm 34$. By way of example of its application to the conditions of this experiment, an indicator-crop planting of sufficient magnitude (6), grown in midsummer under optimum conditions for growth and for infection, giving a reading of say 100 galls per plant, would promise a yield of $-39.5 \times 10 + 1242 \pm 34$, or 847 ± 34 pounds per plot, or, multiplying by $\frac{43560}{900}$

and dividing by 2000, 20.469 ± 1.122 tons to the acre. Had the gall count averaged only 20, or 1/5 as heavy infestation, the yield indicated would have been 1163 ± 34 pounds per plot, or 28.106 ± 1.122 tons per acre. This represents an increase in yield of better than 37 per cent correlative with the improved condition with regard to nematodes.

Naturally, this equation can not be applied generally in estimating yields, for every locality varies in many other yield-influencing factors besides nematode infestation of the soil. Even in the same locality, others of such factors might vary independently of the nematode population to such an extent as to outweigh the nematode factor. It is to be presumed in the experiment just described that the treatments may have reduced other detrimental influences aside from nematodes, and such reductions may have had their influence on yield, as well. Another type of nematode control not influencing such other factors in like degree might bring nematode infestation down to points equal to those here shown without other beneficial effect, in which case yields would not be influenced so greatly. It goes without saying that it is unsafe to rely upon the results of a single such experiment as this for governing field practice. Nevertheless, since the chemical treatments described were the only variables introduced, all other conditions in all plots being as nearly identical as possible under field conditions, considerable stress can be laid upon the reliability of the results reported.

Results of Simultaneous Single-row Tests with Cyanogas and Potassium Xanthate.—Immediately after the installation of experiment 2 as a Latin Square experiment, there being still space for several more rows of the same heavily infested land, a portion of such space was devoted to tests of Cyanogas and potassium xanthate with a view to determining their relative efficiency at different rates of application. The Cyanogas, in granular form, was applied by hand in furrows cut with a plow down the center and on either side of the plant bed, 3 lines in all. The potassium xanthate was similarly applied but with the addition of an equal quantity of acid phosphate, as supplied by the fertilizer trade, in accordance with the recommendations of deOng and Tyler (3). The soil in this section is decidedly on the acid side of neutrality (pH 4 to 5), and was not dry, as shown by the good growth of indicator plants without irrigation; so conditions should have been favorable for best results with this chemical. In both cases, the furrows were filled by cultivators immediately after application of the chemicals, and mulching paper covers were applied as for regular planting.

Rates of application, together with results of treatments in terms of average weight per fruit harvested, are given in table 8.

Casual observations made from time to time during the growth of the plants disclosed definite superiority in plant growth over near-by nontreated

TABLE 8.—*Pineapple-yield data on single-row treatments with Cyanogas and potassium xanthate*

Row	Treatment	Rate per acre in pounds	Number of fruits harvested	Average weight per fruit
43	Cyanogas	870	155	3.20 ± .040
44	"	580	197	3.11 ± .037
45	"	1164	193	3.13 ± .034
46	Xanthate	500	180	3.15 ± .039
47	"	340	167	2.89 ± .043
48	"	680	181	2.97 ± .040
	Checks	(of experiment 2)		2.74 ± .008

rows and over the controls of experiment 2. The yield results show definitely significant increases in weight per fruit over the controls, except in the case of the lightest xanthate treatment. The increases are not sufficient, however, to justify their consideration in comparison with those shown for chloropicrin in experiment 2. From a superficial examination it would appear at once that the costs of the applications are such that their use would be profitless.

DISCUSSION OF EXPERIMENTAL RESULTS

Since the technique of application of chemicals was definitely better in experiment 2 than in 1, without increase in costs, and the results obtained better and probably more significant, the discussion of the economic considerations is confined to the results from experiment 2. Reexamination of the final results in yields per plot (basis m, table 7) discloses definitely increased yields from the chloropicrin applications over the controls. Taking treatment B, 150 pounds of chloropicrin per acre, as a basis of comparison, the increase is 413.5 pounds per plot. Increasing this by 4.44 per cent (because of the removal of this proportion of the plants for earlier examination) the increase to be expected on the field scale is 431.8 pounds. This amounts to 10.435 tons per acre. A conservative estimate of the field value of fruit, based upon the average through 20 years, is 1 cent per pound. The cost of 150 pounds of chloropicrin at 80 cents per pound is $120.00, and the cost of application about $5.00 per acre. With these figures as a basis, the following calculation can be made:

Value of 20870 pounds increase at 1 cent per pound	$208.70	
Cost of chloropicrin treatment	125.00	
Profit arising from the treatment		$83.70

This profit is in plant crop tonnage alone. As additional credits to the chloropicrin should be added 2 items: (1) The higher grade of the fruit

throughout because of the much larger proportion of first-grade fruits on the basis of weights alone (average weight 3.912 pounds as compared with 2.744 for the checks); and (2) the fact of the stand of plants still being in excellent condition after plant crop and, therefore, capable of producing one or more ratoon crops, whereas the check-plot stands were almost worthless in that respect. It would be difficult to estimate the economic value of these additional credits without actual measurements, but they are certainly worthy of consideration. On the basis of plant-crop yields alone, however, the treatment would appear to be justified when it is necessary to plant fields known to be heavily infested with nematodes. Different estimates for basic costs and values would give different figures for profits, but those used can not be far from the average. The feasibility of such treatments as regular field operations under heavy infestation conditions is a matter that can be determined only by consideration of all the economic factors involved.

Similar treatment of the carbon bisulphide data shows a considerably smaller margin between costs and profit from the increased yield, leaving the advantage decidedly in favor of the chloropicrin treatment. The same applies to the Cyanogas and potassium xanthate treatments.

SUMMARY

Two field experiments on the control of the root-knot nematode *Heterodera marioni* in heavily infested Hawaiian pineapple fields, incorporating the use of chloropicrin and certain other chemicals, were conducted in 1931 and 1932. Both were installed with the Latin Square arrangement of plots in order to eliminate soil heterogeneity in infestation. The chemicals were inserted into well-prepared beds that were immediately covered with the usual tar-impregnated, pineapple mulching paper.

Measurements of the effectiveness of the treatments were made by gall counts on cowpea indicator-plant roots, studies on excavated random selected pineapple plants, and, finally, by yield records. In experiment 1, chloropicrin was injected through holes in the paper cover, which were then plugged with soil. In spite of this inefficient confinement of the gas, the following significant benefits resulted from the treatments:

97 pounds per acre, 14 per cent yield increase.
130 pounds per acre, 94.5 per cent reduction in nematodes, 26 per cent yield increase.
160 pounds per acre, 94.4 per cent reduction in nematodes, 31.4 per cent yield increase.

In experiment 2, gas confinement was more efficient by virtue of unbroken paper-mulch covers, throughout. In this case, a comparison with

carbon bisulphide was incorporated. Significant results, in condensed form, are as follows:

C Cl_3NO_2 150 lbs., nematode reduction 83 per cent, yield increase 52.2 per cent.
C Cl_3NO_2 170 lbs., nematode reduction 90 per cent, yield increase 52.3 per cent.
C S_2 750 lbs., nematode reduction 48 per cent, yield increase 29.3 per cent.

Additional data on effects of the treatments on various features of pineapple plant growth and root gall development are tabulated as well. A correlation of $-.852 \pm .0265$ is shown between the original indicator-plant root-gall count and pineapple yields, for the 49 plots of experiment 2. This would appear to establish a definite and highly important detrimental influence of a high initial nematode population on pineapple yields. Definite superiority of chloropicrin at 150 pounds per acre (as applied in plant beds only) over carbon bisulphide at 750 pounds, is shown.

With chloropicrin at 80 cents per pound and a valuation of 1 cent per pound for pineapples, a substantial profit ($83.70 per acre in plant crop alone) from the use of chloropicrin in badly infested fields is established.

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THE RASPBERRY WHITE-BUD DISEASE AND ITS RELATION TO BITTER ROT OF APPLES[1]

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The occurrence of a disease on red raspberries caused by a Glomerella similar in many respects to the bitter-rot fungus suggested the importance of determining its true identity. This disease was of particular concern because apple bitter rot is seldom found in the vicinity of Urbana, Illinois, or north of the latitude of Springfield of the same State (2). Yet, some raspberry plantings were severely infected within this area, by an apparently related fungus. Because of its potential importance to the apple and raspberry industries, the problem of determining the relationship of the raspberry disease to apple bitter rot was undertaken in this investigation.

NATURE OF THE DISEASE

Name.—Due to the whitish gray color of the infected tissue and the fact that the lesions usually occur around the lateral buds this disease has been given the common name "white bud."

Symptoms.—Raspberry white bud is first evident in midsummer as a dark brown discoloration advancing from the vicinity of the bud on the young canes. A few cases have been observed where exposed surfaces on the petioles, near the base, became infected, but generally the fungus seems unable to infect through the weathered epidermis (Table 4). The lesion gradually turns whitish gray following leaf fall, when numerous acervuli are produced, appearing as little black dots over the surface of the lesions. In a mature lesion the bud is usually surrounded by infected tissue. The infection may proceed up the leaf petiole, turning it whitish gray at the base. The leaf falls prematurely and the lateral bud usually dies. It may be killed by direct invasion of the fungus or indirectly through death of the phloem and cortical tissues of the cane to which the bud is attached. The lesions seem always to be localized at the point of union of the cane and the leaf petiole on the shoots (Fig. 1). No leaf infections or infections of second year wood have been found in this study. The disease is most evident during the dormancy of the infected canes. Schwarze (11) reports an abundance of perithecia on the lesions in the spring. Only a few immature

[1] Contribution from the Department of Horticulture, University of Illinois, Urbana, Illinois. The writer wishes to acknowledge his appreciation to Dr. H. W. Anderson and the late Dr. F. L. Stevens for many helpful suggestions and criticisms in carrying out the experiment and preparing he manuscript; to Dr. A. S. Colby for furnishing raspberry varieties for the experiment.

ones have been found under field conditions in Illinois, but mature ascospores have been produced in the laboratory on bean-pod agar.

Host Relationships.—An opportunity was afforded to study the relation of the disease to varieties of raspberries and apples by the occurrence of the disease in the extensive raspberry variety planting on the university farm. Many apple varieties susceptible to bitter rot (Anderson 1) were directly adjacent to the diseased raspberries. This is of significance, since

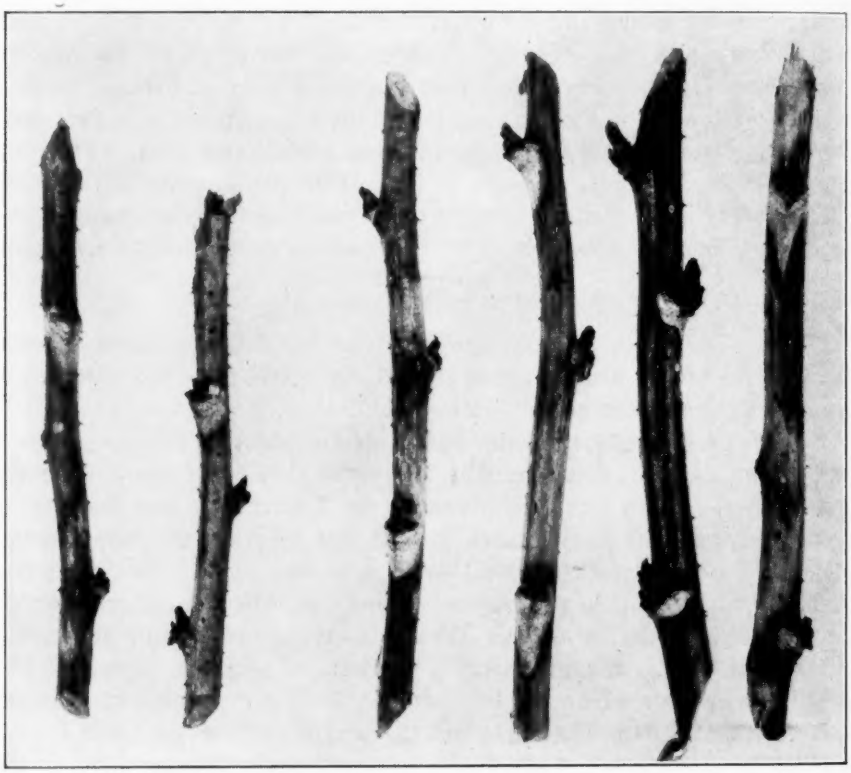

FIG. 1. Raspberry canes, Erskine Park variety, with natural infections by *Glomerella rubicola.*

it will be shown later that if wounds are present to allow its penetration, the white-bud fungus will cause typical bitter rot of apples.

When the fungus is present on the Erskine Park raspberry, nearly all buds are killed. The following varieties usually show between 25 per cent and 50 per cent of their buds killed: June, Perpetuelle de Billiard, Chief, King of Market, Idaho, Newburgh, Jackson, Spineless, and Thornber. Light infections (less than 25 per cent bud kill) may occur on the following varieties: Geneva Station Nos. 2491, 2568, 2584, 1235, Baumforth's Seed-

ling, Victory, Owasco, Rathman, Latham, Fewthorn, and Cayuga. Although 110 varieties of apples were growing immediately adjacent to the diseased raspberries, no bitter rot was found on them.

Economic Importance.—Fortunately the disease is of no great importance on leading commercial varieties, but because of the constant introduction of new raspberry varieties, it may ultimately be of general concern. In this State it has been found only on red raspberries. It appears to have been reported from Canada, New Jersey, and Illinois, but it is undoubtedly present in other States. It could easily be confused macroscopically with raspberry spur blight, *Didymella applanata* (Niessl) Sacc.

Cause of the Disease.—Many typical white-bud lesions from different raspberry varieties were examined in the laboratory. The small black dots, so general on mature lesions, were typically the acervuli of a Colletotrichum. The conidia measured 11–18 μ by 3–7 μ (Fig. 3), and setae were usually present in abundance on infected canes. No mature perithecia were found on the diseased canes, although several immature ones were observed. They are produced on the same sori with the acervuli. The fungus seemed to be identical with *Glomerella rubicola* (Ston.) S. & S.

EXPERIMENTAL COMPARISONS OF APPLE BITTER ROT AND RASPBERRY WHITE BUD

Since a study of the literature of this species indicated some confusion regarding its identity and possible relationship to the bitter-rot fungus, a comparative study of the two organisms was undertaken.

Cultures Used.—The raspberry white-bud fungus was secured by the poured plate method from acervuli from typical lesions. All cultures from different diseased varieties appeared identical. The culture of *Glomerella cingulata* (Ston.) S. & S. used in the following comparisons was obtained from F. L. Stevens, who isolated it recently from diseased apples sent from southern Illinois.

Cultural Studies.—Comparisons of the apple bitter-rot and the white-bud fungi were made on various culture media. The cultural characters are listed in table 1 and shown in figure 2. It may be seen from table 1 that the two fungi differ culturally in several respects, but whether or not these variations are within the limits of variability shown to exist for *Glomerella cingulata* by other workers can not be definitely determined by so limited a comparison. Certainly the differences are sufficient to merit specific rank in groups of more stable fungi. The ascigerous stage was not produced in culture by either fungus when subjected to the ultra-violet ray treatment of Stevens (13), which was found so successful with some strains of the bitter-rot fungus, but it did stimulate acervuli production by the bitter-rot fungus. The aerial growth of both fungi was decreased mark-

edly by this treatment. The perfect stage of the white-bud fungus was produced on bean-pod agar after 6 weeks incubation at room temperature and agrees in general with descriptions of *Glomerella cingulata* as well as the available descriptions of *Glomerella rubicola.*

Ascospore measurements of the raspberry Glomerella show that they fall within the range of the measurements reported for *Glomerella cingulata.* This fact is not especially significant, since the ascospore measurements of the latter species as reported by various investigators vary as much

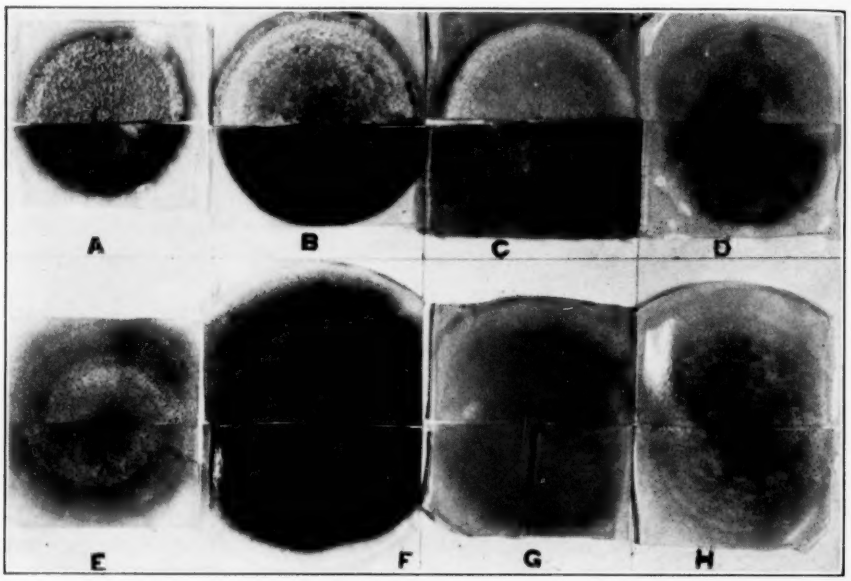

FIG. 2. Top and bottom views of cultures grown for 10 days at room temperature on various agars. A–D. Glomerella from Rubus. E–H. *G. cingulata.* A and E, on prune; B and F, on 2 per cent potato dextrose; C and G, on bean pod; and D and H, on corn meal.

as 16 μ in length and 5 μ in diameter. Spore graphs of the conidia of these two fungi are presented in figure 3. The conidia of the white-bud fungus are slightly longer than those of *Glomerella cingulata.* Again it is difficult to properly evaluate this difference since the literature contains measurements of the conidia of *Glomerella cingulata* much larger than those found by the writer. Another point of interest, but of little morphological value, is the fact that setae are present in abundance in the acervuli of the white-bud fungus as it is found on the infected canes, but have not been observed when this fungus produces acervuli on inoculated apples. Also, a few cases of the production of setae on inoculated apples by the bitter-rot fungus have been observed in this study. That setae are of little value in

TABLE 1.—*Cultural differences of the Glomerella from Rubus and G. cingulata from apple after 10 days' incubation at room temperature*

Media	Color of Cultures (Ridgeway (9))		General Habit	Spore Habit
	Below	Above		
A[a]—Prune *G. rubicola*	Garnet brown (I–k) to Hermosa pink (I–f) Margin white—very variable	Whitish-pink Variable	Fluffy growth above media, 1½ in. growth in 7 days—rudimentary acervuli	Prolific sporulation mostly above the medium
E—*G. cingulata*	Pale olive gray (LI–f) Light around edge	Whitish-gray with blackish streaks	Very slight aerial growth—great abundance of acervuli	"
B—Potato dextrose 2% *G. rubicola*	Hazel (XIV–k) narrow white edge	Pink and fluffy	Fluffy growth with much piling up—2″ in 7 days No acervuli	Prolific sporulation
F—*G. cingulata*	Light buff (XV–f). Along edge—nearly solid black with buff spots	Same—very little aerial growth	Very great acervulus production	"
C—Bean pod *G. rubicola*	Light buff (XV–f)	White and fluffy	No acervuli. Fluffy aerial growth common	Sporulation medium. (Perithecia produced after 6 weeks)
G—*G. cingulata*	Light buff (XV–f)	Light buff to white on margin	No acervuli produced	Sporulation heavy
D—Corn meal *G. rubicola*	Etruscan red (XXVII–b in center to olive buff (XL–d) on margin	Fawn color (XL–b) with spots of Etruscan red	Little tufts of aerial mycelium growth mostly in media. No acervuli	Sporulation medium
H—*G. cingulata*	Pale neutral gray (LIII–d)	Same	Growth mostly in media. Acervuli abundant	"

[a] Letter corresponds to letters on figure 2.

the taxonomy of fungi has been demonstrated by other workers. In brief it may be said that so far as the two fungi under consideration are concerned, they show rather striking cultural differences; but since the general group of anthracnose fungi are known to vary considerably, both culturally and morphologically, the variations encountered may not be sufficient to regard them as distinct. It seems, therefore, that the only hope of reliable separation of these two fungi is in their host relations.

Inoculation Tests.—In table 2 all of the apples were inoculated by Huber's (6) method. The columns marked "Positive results" indicate the

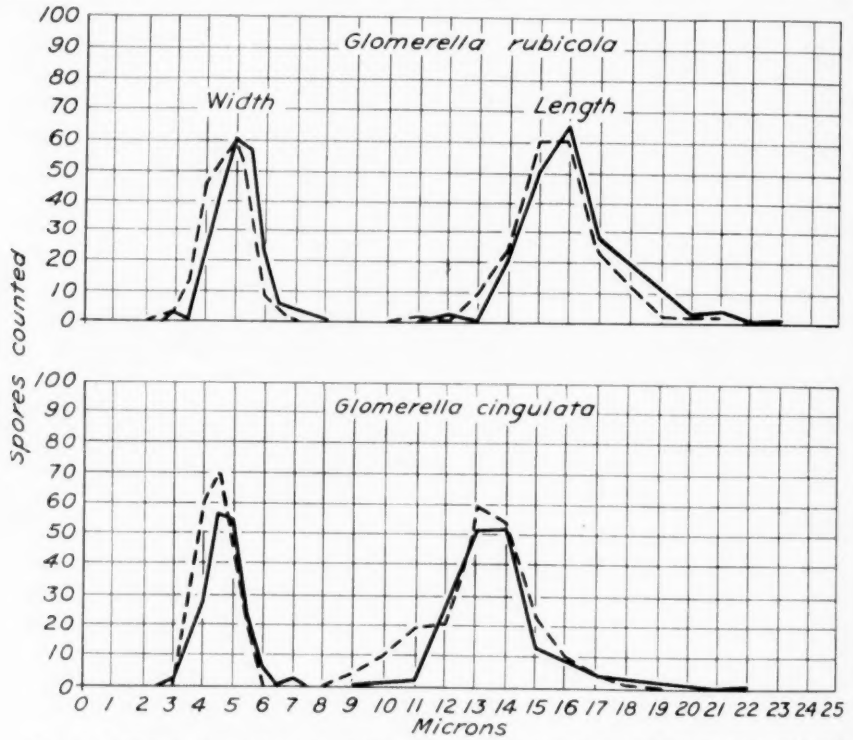

FIG. 3. Distribution of spore widths and lengths for *G. cingulata* and *G. rubicola*. Solid line, grown on 2 per cent potato-dextrose agar. Broken line, grown on inoculated apple.

number of successful inoculations. No contamination was in evidence. The methods of this experiment were fundamentally the same as those followed by Dodge (4), when he successfully produced lesions on raspberry canes inoculated with a strain of *Glomerella cingulata*. He also produced typical apple bitter rot on apples inoculated with the fungus isolated from diseased canes. The results of cross-inoculation experiments of this investigation are listed in table 2. Ben Davis and Grimes apples and the Erskine Park raspberry were used in this experiment.

TABLE 2.—*Cross-inoculations with raspberry white-bud and apple bitter-rot fungi. Inoculated apples held at room temperature. Raspberries were inoculated under greenhouse conditions. Canes inoculated by placing bits of the fungus growth from culture media into wounds made with a scalpel*

Fungus used	Apples			Raspberries	
	Varieties used	Number inoculated	Positive results	No. of canes inoculated	Positive results
White bud	Ben Davis	30	30	20	20
White bud	Grimes	30	30	20	19
Bitter rot	Ben Davis	30	30	20	0
Bitter rot	Grimes	30	30	20	0
Checks	Ben Davis	5	0	5 wounds	0
	Grimes	5	0		

Both fungi caused typical bitter rot of apple when inoculated into the flesh of the apple (Table 2, Fig. 4, A and C). *Glomerella cingulata,* the bitter-rot fungus, did not cause lesions of any type on inoculated canes of the Erskine Park raspberry while the white-bud fungus did in every case but one (Fig. 5). The environmental conditions were the same for both fungi throughout the experiment. These results do not confirm the work of Dodge (4), but it is possible that he was using a different strain of apple bitter rot. The variety of Rubus used might also account for the difference in results. Shear and Wood (12) have regarded the ability of a fungus to produce typical bitter rot of apple when inoculated into the flesh as sufficient evidence for placing several species of Glomerella in the single species *Glomerella cingulata.* There is a question as to whether positive wound inoculations into mature apples constitutes experimental evidence of sufficient value for regarding the fungus as naturally parasitic on apple. Since the Glomerella from Rubus and the Glomerella from apple both caused typical bitter rot of apple, they might at least be regarded as strains of *Glomerella cingulata.* It is evident, however, that the mature apple contains an abundance of readily available sugars, moisture, and other chemicals which are necessary for the growth requirements of a given group of fungi. *Glomerella cingulata* causes great injury to apple crops because the fungus is able to penetrate the unbroken epidermis of the fruit. The fact that one fungus will enter an apple through an unbroken skin and another will not undoubtedly offers a more reliable basis of possible separation than the information gained when the fungi to be tested cause rotting after inoculation by wounding the apple. Evidence of this nature, if used by itself, might indicate nothing more than strains of a given fungus, but if supplemented by other recognized procedures might play a more important diagnostic rôle.

In order to determine whether the fungi under consideration could penetrate the unbroken epidermis of mature apples and cause typical apple bitter rot, the following experiment was carried out. Spores of the Glomerella from Rubus and from *Glomerella cingulata* were atomized onto apples

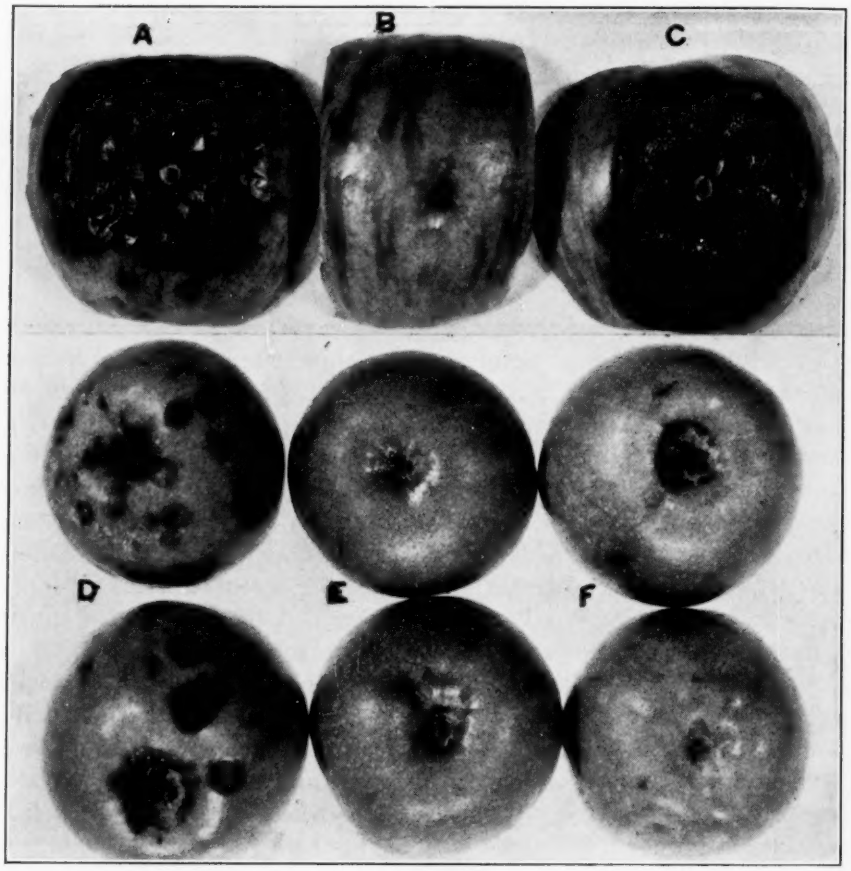

FIG. 4. A to C. Ben Davis apples. A and C. Typical bitter rot produced by artificial inoculations into the pulp with *Glomerella cingulata* and Glomerella from Rubus, respectively. B. Check. D to F. Grimes Golden apples after atomizing the uninjured fruit and keeping two weeks in a damp chamber at room temperature. D. With spores of *G. cingulata*. E. With sterile distilled water. F. With spores of Glomerella from Rubus.

that had been previously sterilized with 1–1000 $HgCl_2$ and rinsed in sterile distilled water. The apples were then placed in damp chambers and held at room temperature for ten days. The results of this test are given in table 3 and illustrated in figure 4, D to F.

FIG. 5. Young Erskine Park shoot showing lesions caused by Glomerella from Rubus 4 weeks from time canes were inoculated by needle puncture.

The results of this experiment show conclusively that *G. cingulata* will penetrate the unbroken skin of Grimes apples and that the Glomerella from Rubus will not, under the environmental conditions of this test.

A test similar to the one just described was run to determine if the fungi would penetrate the unbroken epidermis of young vigorous raspberry shoots, and, if so, whether entrance was always through the unweathered tissue at the junction of the leaf petiole and the cane, as is evidently the case under field conditions. Table 4 gives the results of these tests.

Apparently the tissue in the vicinity of the lateral bud is the only place where infections take place, unless wounds are present on the canes. *Glom-*

TABLE 3.—*Surface of Grimes apples atomized with spore suspensions of Glomerella from Rubus and Glomerella cingulata to test ability of fungi in question to enter unbroken epidermis of apples. Incubated in damp chambers at room temperature ten days*

No. of apples atomized	Spore suspension	No. of positive infections[a]	Location of lesions
10	*G. cingulata*	93	General
10	G. from Rubus	1	Calyx end
10	Sterile water	0	Centered at worm puncture

[a] Reisolations made from typical lesions and compared with cultures used to make spore suspensions.

erella cingulata did not cause any disease on Erskine Park. The experiments show that the Glomerella from Rubus is capable of causing the white-bud disease by infections through the uninjured epidermis of raspberry canes.

TABLE 4.—*Atomizing spore suspensions of G. cingulata and Glomerella from Rubus on vigorous young shoots of Erskine Park raspberries. Plants held in inoculation chambers for 48 hours after being atomized and then grown under greenhouse conditions*

No. of canes	Spore suspension	No. of positive infections[a]	Location of lesions
5	Glomerella from Rubus	18 buds out of 41	At junction of petiole and cane—one petiole infection
5	*G. cingulata*	None	—
2	Sterile water	None	—

[a] Fungus reisolated from lesions in a few typical cases.

DISCUSSION

Taxonomic Status of Raspberry White-bud Fungus

A Gloeosporium was collected from the leaves of *Rubus strigosus* by Nuttall in West Virginia (1895). It was distributed by Ellis and Everhart as No. 3468 of North American Fungi under the name *Gloeosporium rubicolum*. Later it was described under this name by Millspaugh and Nuttall (7, p. 123) who credited it to Ellis and Everhart. Specimens of apparently the same collection were sent to Miss Stoneman (14, p. 108–110) who had evidently overlooked both the exsiccati specimen and the previous de-

scription by Millspaugh and Nuttall (7, p. 123), since she changed the name to *Colletotrichum rubicolum* E. & E. Because setae were evidently present in the material she examined, it is possible that she purposely changed the generic name. Later, Stoneman (14) found the perfect stage in culture and described it as *Gnomoniopsis rubicola,* which was changed by Von Schrenk and Spaulding (9, 10) in 1903 to *Glomerella rubicolum* (E. and E.) S. and S. Another Gloeosporium was described by Ellis and Everhart from *Rubus villosus* as *Gloeosporium rubi.* Since this fungus occurs on blackberries and since the spores are much narrower than those of *Glomerella rubicola,* it is evidently a different species. Shear and Wood (12) regard *Gloeosporium rubi, Glomerella rubicola* and *Glomerella cingulata* as synonomous. They imply that Stoneman (14, p. 108–110) regarded the imperfect stage as *Gloeosporium rubi,* but, according to the article they cite, she reports the imperfect form as *Colletotrichum rubicolum.* Both Stoneman (14) and Atkinson (Stoneman (14) Page 81) regard *Gloeosporium rubi* as distinct from *Glomerella rubicola* (*Colletotrichum rubicolum*). In 1914 Schwarze (11) described and figured a disease on the stems of raspberries, which was ascribed to *Glomerella rubicola* (Ston.) S. and S. and listed as the imperfect stage as *Colletotrichum rubicolum.* Since this fungus was found on canes while the original species occurred on the leaves, it is presumed that he based his identification on morphological details. Judging from his account of the disease it is identical with the one studied in these tests. In 1908 Edgerton (5) successfully rotted apples with a Colletotrichum taken from raspberry stems. From available descriptions it is impossible to determine whether or not Edgerton regards the form on stems as being identical with the form described on leaves by Stoneman (14). Shear and Wood (12) regarded the experiment of Edgerton as sufficient evidence to consider the apple bitter-rot fungus and the raspberry Colletotrichum as identical. Dearness (3, p. 68) lists *Glomerella rubicola* as being present in Canada, but does not give a description of the disease caused by it. In 1927 Dodge (4) published the first work with sufficient experimental evidence to justify the conclusion that the form he described on the raspberry is the same or very closely related to the apple bitter-rot fungus. His descriptions and figures, as well as the experimental evidence presented lead to the conclusion that the form worked with is not the same as the one under consideration. Other Gloeosporia have been described on *Rubus* spp., but are not included in this discussion because their morphological characters eliminate them as possible synonyms.

CONCLUSION

From the foregoing investigation it is believed that the white-bud fungus is a distinct species and should not be regarded as a strain of the bitter-rot

fungus. While according to Dodge (4) strains of *Glomerella cingulata* are capable of infecting raspberries, the symptoms produced by this fungus are not typical of those produced by the white-bud fungus. *Glomerella cingulata* from apple bitter rot infects sound apples through the unbroken epidermis, while the white-bud fungus does not; consequently the pathogenic properties of the two are distinct. There are also variations in cultural and morphological characters that are in themselves of less importance in this variable group, but when taken in conjunction with the decided difference in pathogenic properties may be regarded as significant. Since the name, *Glomerella rubicola*, has been given to a fungus producing similar symptoms on raspberries to those herein described and since the morphological characters agree, it is thought best to retain this name for the white-bud fungus.

SUMMARY

1. Raspberry white bud is a disease found only on red raspberries in Illinois. On certain varieties it is very injurious.

2. The white-bud fungus is distinct in pathogenicity, symptoms, and in cultural and morphological characters from the strain of *Glomerella cingulata* generally present in Illinois.

3. The white-bud fungus will cause typical bitter rot of apples if inoculated into them.

4. In inoculation tests on Erskine Park the white-bud fungus gave positive results, while the strain of *G. cingulata* used did not.

5. *Glomerella cingulata* spores atomized on apples infected them through the unbroken epidermis. The Glomerella from Rubus did not infect apples under the same conditions.

6. The Glomerella from Rubus caused the white-bud disease of Erskine Park by direct penetration of the epidermis in the vicinity of the buds, but *G. cingulata* under similar conditions did not.

7. Evidence is presented to justify the conclusion that the white-bud disease is caused by *Glomerella rubicola* (Ston.) S. & S.

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THE QUALITY OF LINT AND SEED FROM COTTON PLANTS WITH PHYMATOTRICHUM ROOT ROT[1]

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In a separate paper,[2] the writers have discussed the yield secured from cotton plants known to have succumbed to root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, at various periods during the season. The present paper is concerned with the effect of the disease on the *quality* of the lint and seed picked from these same plants.

MATERIAL AND METHODS

The cotton lint and seed were picked in 1931 from Startex (Texas Station No. 7000) plants, growing in an experimental field at College Station, Texas. At weekly intervals during the season, plants killed by root rot or wilted from advanced injury by the disease were marked with small stakes bearing letters to indicate the dates when the plants had apparently succumbed. On September 10 and 28, the cotton from each plant was picked into a separate envelope, dried in the laboratory for one month, and ginned and weighed separately for each plant. Summaries of the yields obtained have been given in the previously mentioned paper.[2] The results for the individual plants have been considered together in lots consisting of plants that succumbed to root rot within approximately weekly intervals during the season.

The length of lint was determined prior to ginning. Where possible, 25 individual seeds were selected for each lot, each seed being taken from the central seeds of a lock from a different plant. The 25 plants selected for this purpose constituted "stratified" samples and were selected to include for each lot proportionate numbers of the plants bearing 1, 2, 3, and so on bolls per plant, rather than merely random samples from the mixed crop. In the lots that succumbed to root rot early in the season there were less than 25 plants that bore cotton, and several seeds were, therefore, selected from each plant of these lots. The lint was measured in millimeters, as summarized in table 1.

Counts were made of seeds from 10 representative plants of those lots in which 10 or more plants bore cotton. These counts, together with the data on yields of these plants, furnished the basis for caculation of lint indexes, as given in table 2.

[1] Published with the approval of the Director as Contribution No. 295, Technical Series, Texas Agricultural Experiment Station.

[2] Ezekiel, Walter N., and J. J. Taubenhaus. Cotton crop losses from Phymatotrichum root rot. (In press). Jour. Agr. Res. 49: 1934.

TABLE 1.—*Lengths of cotton lint produced by plants that succumbed to Phymatotrichum root rot at different periods during the summer of 1931. (Determined from 25 individual measurements of combings of lint from different plants, except only 10 measurements made for lot 2)*

Lot No.	Interval during which plants succumbed to root rot	No. of plants from which lint was measured	Range of individual measurements	Coefficient of variability of measurements[c]	Mean length and probable error
			mm.	*mm.*	*mm.*
1[a]	to June 30	5	12–25	17.2	21.84 ± .52
2[b]	July 1 to July 7	1	10–20	26.0	15.00 ± .88
3	" 8 to " 15	6	9–26	24.8	21.04 ± .72
4	" 16 to " 22	13	17–28	14.6	23.20 ± .47
5	" 23 to " 30	22	12–33	20.9	23.52 ± .68
6	" 31 to August 4	25	17–29	14.1	24.24 ± .47
7	August 5 to August 11	25	20–28	10.2	24.24 ± .34
8	" 12 to " 18	25	17–28	11.2	23.76 ± .37
9	" 19 to " 25	25	22–27	6.0	24.44 ± .20
10	" 26 to September 3	25	19–27	8.4	22.88 ± .26
11	September 4 to September 10	25	17–28	10.6	23.84 ± .35
12	Normal plants	25	15–30	13.2	23.80 ± .43

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.
[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.
[c] The coefficient of variability = 100 times the standard deviation, divided by the mean.

TABLE 2.—*Lint indexes of cotton produced by plants that succumbed to root rot at different periods during the summer of 1931. (Mean values in this table refer not to all plants of various lots, but only to the 10 or fewer plants used in calculation of indexes)*

Lot No.	Interval during which plants succumbed to root rot	No. of plants for which indexes calculated	Mean number of seeds per plant	Mean weight per seed, gm.	Mean percentage lint	Lint indexes	
						Range of indexes of individual plants	Mean per lot
1[a]	to June 30	5	38.8	0.07	42.4	1.5– 6.8	4.6
2[b]	July 1 to July 7	1	27.0	0.08	42.1	5.9	5.9
3	" 8 to " 15	6	46.6	0.04	48.7	1.3– 3.5	2.4
4	" 16 to " 22	10	41.4	0.03	42.5	1.3– 2.7	2.0
5	" 23 to " 30	10	83.9	0.04	40.1	1.5– 4.6	2.7
6	" 31 to August 4	10	62.5	0.06	41.0	2.4– 5.6	3.7
7	August 5 to August 11	10	80.2	0.07	36.8	1.7– 8.4	4.0
8	" 12 to " 18	10	72.4	0.07	40.1	2.6– 8.2	4.7
9	" 19 to " 25	10	81.4	0.10	40.5	4.8– 8.8	6.7
10	" 26 to September 3	10	74.4	0.12	39.8	5.2–10.5	7.9
11	September 4 to September 10	10	64.4	0.11	40.1	4.1– 8.5	7.3
12	Normal plants	10	87.0	0.10	41.0	4.5– 8.9	6.9

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.

[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.

After ginning, the lint and seed from the individual plants were massed together into the lots representing the total crop produced by plants succumbing to root rot at the various periods during the season. The combined lint samples were given commercial classing by J. G. Powers (Table 3) of the Department of Textile Engineering of the Agricultural and Mechanical College of Texas. Microscopic study of these same cotton fibers was made later and is presented as the third paper of this series.[3]

The cotton seed from the various lots was divided into 2 portions. One portion was used for germination tests, and another was analyzed by the Division of Chemistry, Texas Agricultural Experiment Station. The results of these analyses are given in table 4.

RESULTS

Length of Lint

The measurements summarized in table 1 indicate a slight but definite effect of root rot on the length of lint. The lint from the 5 lots of plants that succumbed prior to July 31 averaged only 20.9 mm., as compared to 23.9 mm. average for the other 6 lots and 23.8 mm. for lint from the normal plants. Omitting the scanty data for lot 2, the mean lengths for the other 4 lots succumbing prior to July 31 averaged 22.40 mm. $\pm$ .30 mm., as compared to 23.90 mm. $\pm$ .14 mm. for the other 6 lots of root-rot plants. The difference between these means, 1.50 $\pm$ .33, while slight, amounted to about $4\frac{1}{2}$ times the probable error and was thus statistically significant. A possible indirect effect of root rot on the length of the ginned fiber will be discussed below.

Lint Index

A measure of the abundance of lint on the seeds borne by these plants is furnished in table 2, in the form of the "lint index" for 10 representative plants of each lot. The lint index is the weight in grams of fiber produced by 100 seeds. It was calculated from the data for individual plants by multiplying the weight of 100 seeds by the percentage of lint and dividing by the percentage of seeds. Beginning with very low mean indexes of 2.4 and 2.0 in lots 3 and 4, the indexes increase progressively to 7.9, 7.3, and 6.9 in lots 10, 11, and 12. Lots 1 and 2 are apparently aberrant, with indexes respectively of 4.6 and 5.9; but much of the yield in these lots was on plants that recovered and were still green when the first picking was made. Early attack of root rot, therefore, not only reduces the yield of lint and seed per plant and per boll, as reported in the previous paper, but also reduces the yield per seed.

[3] Stroman, G. N., J. J. Taubenhaus, and Walter N. Ezekiel. Some effects of Phymatotrichum root rot on the microscopic characters of cotton fibers. Phytopath. 25: 126–130. 1935.

TABLE 3.—*Commercial grades of cotton lint produced by plants that succumbed to root rot at different periods during the summer of 1931*

Lot No.	Interval during which plants succumbed to root rot	Grade of staple	Length of staple	Character of staple	Discount from price or premium[c]
			inches		
1[a]	to June 30	Strict Low Middling	31/32	Weak and irregular	65 points off
2[b]	July 1 to July 7	"	3/4	"	175 " "
3	" 8 to " 15	"	15/16	"	75 " "
4	" 16 to " 22	"	13/16	"	125 " "
5	" 23 to " 30	"	31/32	"	65 " "
6	" 31 to August 4	"	15/16	"	75 " "
7	August 5 to August 11	"	1	Slightly irregular	5 " "
8	" 12 to " 18	"	1	Normal	Even
9	" 19 to " 25	"	1+	Weak	5 points off
10	" 26 to September 3	Middling	15/16	Slightly irregular	10 " "
11	September 4 to September 10	"	15/16	"	10 " "
12	Normal plants	"	1	Good character	25 " on

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.

[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.

[c] Stated as points, or hundredths of a cent per pound; 100 points is considered as $5.00 per bale difference in price. By "on" is meant that the stated number of points is to be added to the price of middling 15/16 inch, normal character cotton; and by "off" that the stated number of points is to be subtracted from the basis price of middling.

Root rot apparently had little effect on the percentage of lint borne by plants of the various lots (Table 2). There were slightly higher percentages of lint on plants succumbing to root rot early in the season than on those succumbing later.

Commercial Grade of the Lint

The effect of root rot as possibly influencing the price paid for cotton from root-rot plants was determined by actual commercial grading of the massed samples of ginned cotton (Table 3). Lint from plants that succumbed to root rot up to 2 weeks before the date of picking was graded as Strict Low Middling, while lint from plants that succumbed very late in the season and from the normal plants was designated as Middling. In addition, the staple from most of the early lots was characterized as weak and irregular, while staple from the normal lots and from plants succumbing later in the season was of better character.

The length of staple of the various lots of this ginned cotton (Table 3) did not agree exactly with the earlier measurements of length of lint on the seeds (Table 1). This was to be expected, both on account of the smaller number of measurements necessarily made in the commercial grading, and because ginning may have shortened the fibers of the cotton from root-rot plants to a greater extent than the fibers from the normal plants. In the commercial rating, the 6 lots that succumbed to root rot prior to August 5 were all classed as less than 1 inch long, while 4 of the 6 other lots were classed as 1 inch long.

The premium or discount from the selling price for basis cotton was estimated by the classer in the customary way, largely from the grade and the staple length of these various lots as he had determined them. Differences between the various lots were considerable. The 6 lots from plants succumbing prior to August 5 drew discounts of 65 to 175 points, while cotton from plants succumbing later in the season was given only negligible discounts and the normal cotton was assigned a premium of 25 points. In terms of dollars per bale of cotton, these differences indicate a reduction from the average price of around $30 a bale, received by growers for the 1931 crop, to about $21.25 for cotton of the poorest lot. The cotton from the normal plants should have commanded a price of about $31.25 per bale. The average discount for lots 1 to 6, inclusive, was 97 points, representing a price of about $25 a bale for cotton picked exclusively from plants succumbing to root rot early in the season.

In farm practice, however, the yield from root-rot and normal cotton plants is mixed in picking and ginning. From the portions of the total crop borne by plants of the different lots, it was possible to estimate the theoretical effect of the discounts or premiums assigned to lint of the vari-

ous lots, on the price of a mixture of cotton of all the lots. Thus while the staple of lots 1 to 6, inclusive, was heavily discounted, these lots constituted only 5.6 per cent of the total crop, and would presumably have lowered the grade of the entire crop by only about 21.4 cents per bale. For the entire crop, there would have been a net loss of about $1.15 a bale from the price assigned the cotton from the normal plants. It is obvious that this estimated reduction in the quality of this cotton is not necessarily the same as might have been assigned in actual commercial classing of the cotton picked from the field. The presence in the sample of around 6 per cent of inferior cotton would be considered of varied significance by different cotton classers.

Considering only the cotton harvested from plants with root rot, it may be noted that only about 10 per cent of this total was made up of the inferior staple of lots 1 to 6. However, this cotton was picked from a field, under artificial irrigation, in which root rot had continued to spread at a fairly uniform rate during the entire season. Under dry-farming conditions, on the other hand, the disease may kill many plants in infested fields fairly early in the season, only to be checked soon by dry summer weather. The cotton picked from root-rot plants in such fields would, therefore, consist more largely of cotton from plants attacked early in the season. The lint from the root-rot plants would correspondingly cause a greater average reduction in quality of the mixed crop than would be expected from the data from the irrigated plants as given in the present paper. We may suggest that, for dry-farming conditions, from 10 to 20 per cent of the cotton from root-rot plants might be borne on plants that succumbed to root rot early in the season and, therefore, produced staple of inferior quality. For example, if as much as 80 per cent of the total yield was picked from plants that had succumbed to root rot, only 8 to 16 per cent of the total yield would be expected to be of poor quality staple.

Effect of Root Rot on the Quality of Cotton Seed

Germination test of seeds from 9 of these lots showed a general unfavorable effect from early attack of root rot (Table 4). The 3 lowest percentages of germination were obtained in the seed from plants that succumbed to root rot prior to August 5. Seed from plants that succumbed to root rot after this date germinated nearly, if not quite, as well as those from the normal plants.

Chemical analysis of the dried seeds showed a slight reduction in protein content in seeds from the plants that succumbed to root rot early in the season. Lots 3 to 7, inclusive, all contained less than 24 per cent of protein, while lots 8 to 12 all contained more than 24 per cent. The apparently aberrant results in lots 1 and 2 can be explained, as in other relations, by the fact that much of the yield in these lots was on plants that recovered from the disease.

TABLE 4.—*Results of germination tests and chemical analyses of cotton seed from plants that succumbed to Phymatotrichum root rot at various periods during the summer of 1931*

Lot No.	Interval during which plants succumbed to root rot	Germination of seeds	Chemical composition of dried seeds, percentages of		
			Water	Protein	Fat
		per cent			
1[a]	to June 30	[c]	2.04	23.00	11.44
2[b]	July 1 to July 7	[c]	2.64	24.35	13.48
3	" 8 to " 15	34.0	1.50	22.46	6.86
4	" 16 to " 22	82.4	1.30	21.80	5.88
5	" 23 to " 30	47.0	1.91	20.54	6.22
6	" 31 to August 4	28.5	1.61	23.52	8.28
7	August 5 to August 11	62.5	1.49	23.34	11.34
8	" 12 to " 18	67.3	1.80	24.37	12.62
9	" 19 to " 25	73.3	1.72	24.71	14.38
10	" 26 to September 3	76.5	1.30	24.86	18.10
11	September 4 to September 10	57.0	1.35	24.58	17.22
12	Normal plants	67.0	1.50	24.67	18.20

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.
[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.
[c] Insufficient seed for germination test.

The most striking effect of root rot was on the fat content of the seeds. Seeds of lots 3, 4, and 5 contained only about 6 per cent of fat. With later attack of root rot, the fat content increased progressively to 18 per cent for seeds from the normal plants. The results with lots 1 and 2 were again out of line. It is to be noted that the fat content of the seeds of lots 7, 8, and 9, from plants that succumbed to root rot only 2 to 5 weeks preceding harvest, were lower than with the normal seed, although not so low as with seed from plants attacked earlier in the season.

Weighting the percentage of fat content in seeds of the different lots by the weight of seeds produced on these plants, an average figure of 14.9 per cent was obtained for all the seed from root-rot plants. This is 3.3 per cent lower than the 18.2 per cent fat content of the normal plants. Most of this loss was confined to the seed from plants that succumbed to root rot prior to August 26. The fat content for lots 1 to 9, inclusive, averaged only 11.3 per cent, while in the 2 lots succumbing between August 26 and September 10, the fat content was 17.8 per cent, not greatly different from that of the normal plants.

The average loss of 3.3 per cent fat content, which was a little more than 18 per cent. of the fat content of the seeds from normal plants, applied to the seed from the particular root-rot cotton plants grown in the experimental field, which was irrigated. As was pointed out above, under dry-farming conditions higher percentages of the plants with root rot may succumb during the early part of the season, when reduction in the quality is greater, rather than later in the season. For dry-farming conditions, the seeds from root-rot plants might have the fat content reduced by as much as 18 to perhaps 35 per cent of the percentage of fat in seeds from normal plants.

The price paid to growers for cotton seed is in some places adjusted for the protein and fat content of the seeds. In such areas there would be direct cash losses to growers from the reduced quality of seeds harvested from plants with root rot. In other areas, where the analysis is ignored in setting prices for individual purchases, the lowered value of seeds from root-rot plants would still necessarily affect the average price paid, even if no particular penalty were assigned to cotton seed from a root-rot field.

SUMMARY

Cotton lint picked from plants in an experimental field at College Station, that succumbed to root rot 5 weeks or more prior to the date of picking averaged 21.47 mm. in length as compared to 23.8 mm. for lint from plants that succumbed later and from normal plants.

The average lint index of cotton from plants succumbing early in the season was markedly lower than with cotton of the other lots, indicating

that root rot reduces the weight of lint borne per seed, as well as per plant and per boll.

Lint from plants that succumbed to root rot 3 weeks or more prior to the date of picking was graded one grade lower than cotton from plants that survived until later in the season or cotton from the normal plants.

Despite the definitely lower quality of lint from the plants succumbing to root rot early in the season, there was a relatively small percentage of inferior cotton in the total crop, due to the meager crop borne by the plants that were attacked early. It is suggested that under dry-farming conditions probably not more than 10 to 20 per cent of the lint from plants with root rot would be from plants that succumbed to the disease early in the season and produced staple of inferior quality.

Cotton seed from plants that succumbed to root rot early in the season were lower in germination, slightly lower in protein content, and considerably lower in fat content, than was the case with seed from plants that survived until later in the summer or remained normal until harvest.

It may be concluded that root rot has a considerable effect on the quality of lint and seed from cotton plants. This effect is directly proportional to the earliness of attack. Plants attacked early in the season bear relatively small crops, thus the portion of the total crop that is of low quality will probably rarely exceed 20 per cent of the yield from the plants that are attacked by the disease. The loss to the individual grower from reduction in the quality of the crop, therefore, is much less important than the reduction in the size of the crop that may result from attack of root rot.

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BACTERIAL LEAF SPOT OF CARNATIONS

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The bacterial leaf spot of carnations, *Dianthus caryophyllus* L., caused by *Phytomonas woodsii* is a disease that has been known for many years. Its early history, however, is somewhat confused with several other troubles of this plant. In 1889, Arthur (1) ascribed a spot disease of carnations to bacteria, and subsequently Arthur and Bolley (2) presented a more detailed account of the disease and named the pathogen *Bacterium dianthi,* n. sp. Later Woods (8 & 9), in a series of papers attempted to show that the disease described by Arthur and Bolley was due to insect injuries (stigmonose), that the bacteria were secondary and that *Bact. dianthi,* as described, was not pathogenic. Nevertheless in 1903 Woods (10) did describe a bacterial leaf spot of carnations, but he considered it entirely different from anything hitherto reported. He did not name the causal organism, however, nor did he give an adequate description of it. Smith (7), in a footnote in volume 2 of Bacteria in Relation to Plant Diseases, finally presented a brief and incomplete description of the pathogen and named it *Bact. woodsii,* n. sp. Such is the early history of the disease.

A careful study of the work of Arthur and Bolley leaves one uncertain as to the carnation trouble that they had under observation. Nevertheless, certain of the symptoms reported by them are characteristic of the disease now known to be due to *Phytomonas woodsii.* These workers, furthermore, reported successful inoculation experiments on several species in the genus Dianthus, so they must have been working with a pathogen. *Bacterium dianthi,* the species described by them, was a yellow-pigment producer, and the causal organism of the carnation leaf spot is known to be white. It is, therefore, possible that Arthur and Bolley were working with a mixed culture, a fast-growing yellow saprophyte that obscured the slow-growing *Phyt. woodsii.*

No serious investigation of the bacterial leaf spot of carnations has been undertaken since the above mentioned articles, and no adequate description of the pathogen has been published. It would appear, therefore, from this neglect of the disease that it has been of minor importance. This supposition is not altogether true, however. In certain parts of the country, particularly on Long Island and in the east-central States, the bacterial leaf spot results in serious losses under both field and greenhouse conditions. The disease in the field is very severe during summers of abundant rainfall. It is also troublesome in greenhouses in the early fall when the

weather is cool, wet, and cloudy, since, under such conditions, it is frequently impossible for the grower to keep his plants dry.

Many of the varieties of the carnation grown commercially show marked susceptibility to the leaf spot, while certain varieties have been observed to be resistant. Both Red and White Matchless have been reported from various States as being highly susceptibile. In addition to these varieties, Gregory (5), in Indiana, has found the disease to be extremely severe on Betty Lou and Spectrum. Massey (6), after making observations during an epiphytotic on Long Island in 1928, listed Matchless, Eldora, Rosalind, Scepter, and Spectrum as susceptible and the Enchantress varieties as resistant. The writers during the last 3 years have found the variety Sophelia to be very susceptible, while Spectrum Supreme, Salmon Spectrum, Potentate, and Patrician were found to be moderately susceptible.

SYMPTOMS

Symptoms exhibited by the several carnation varieties susceptible to the bacterial leaf spot are fairly constant except for slight variations in color. The necrotic spots resulting from infection (Fig. 1 A and B) are sunken and of a more or less oval shape with the long axis of the leaf. The centers of the lesions in reflected light are pale brown with purplish concentric zonations, while the outer zone is water-soaked and yellow in transmitted light. Under conditions of high humidity the bacteria may ooze out on the surface of the lesion in a small drop. The spots average 8–12 mm. in length and may include the entire breadth of the leaf. With numerous centers of infection on a single leaf, the spots tend to coalesce.

Several spots ultimately cause the death of a leaf. This killing begins with a yellowing that occurs in advance of the water-soaked margin about the older lesions. The leaf gradually turns a straw yellow, withers, and dies, while the spots retain a rusty brown color.

The progress of the disease on a plant is from the lower leaves upwards. Symptoms always have been observed to appear first on the older leaves under both field and greenhouse conditions. While the bacterial leaf spot is, primarily, a foliage disease, it should be pointed out that in rare cases natural infections have been observed on the stems and flower buds, as well. No evidence has been obtained, however, that the disease is systemic. In cases of severe infection the plants may be killed. In those instances where the infection is less severe flower production may be arrested or curtailed for a considerable length of time.

PATHOGENICITY

Numerous inoculation experiments have been conducted with 3 different isolates of *Phytomonas woodsii* on several varieties of carnations, but, since

100 per cent infection occurred in all cases where conditions were favorable, the data are not presented. The first symptoms of the disease appeared approximately 3 to 4 days after inoculation. In no case, however, was infec-

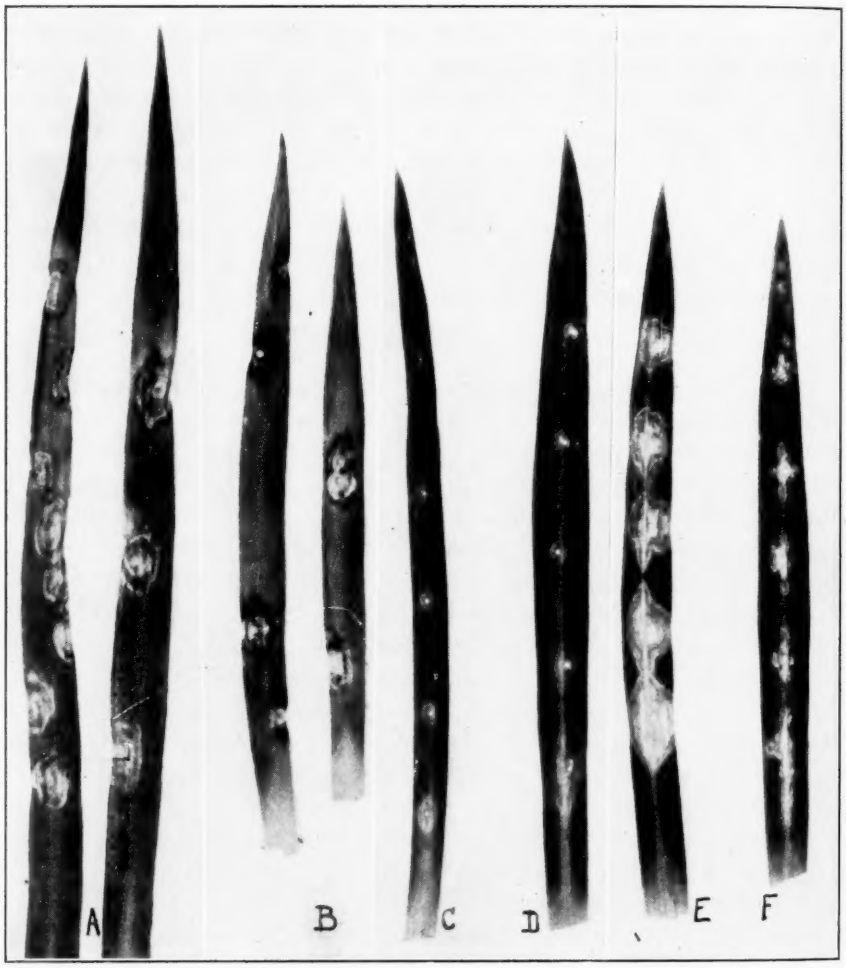

FIG. 1. A and B. Leaf spots resulting from natural infection with *Phyt. woodsii* on the varieties Spectrum (A) and Potentate (B). C to F. Effect of temperature on the development of bacterial leaf spot on leaves of the variety Sophelia. C, 60°; D, 65°; E, 75°, and F, 80° F.

tion obtained without wounds. This finding is at variance with the statements of Woods (10) and Smith (7) who claim that infection can be obtained in the absence of wounds, the bacteria presumably gaining entrance through the stomata. A strenuous, but unsuccessful, attempt has

been made to duplicate the results. Bacteria have been atomized and painted on unwounded leaves, and in some instances the bloom was rubbed free from the treated leaf. Both old and young leaves have been inoculated. Plants under test have been left in a moist chamber several days before and after inoculation. Variations in greenhouse temperature also have been utilized. In 4 experiments, drops of a bacterial suspension have been maintained for at least a week on the leaves of plants under bell

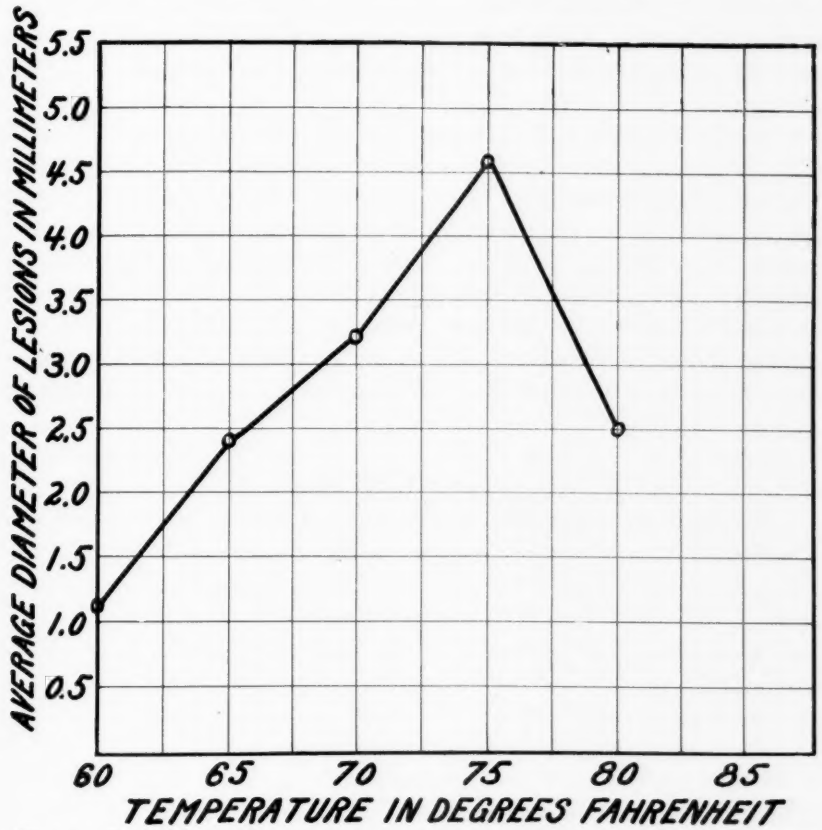

FIG. 2. Effect of temperature on the development of lesions resulting from artificial inoculation of the varieties Sophelia and Laddie.

glasses, but with negative results. In comparable series of experiments using plants whose leaves had been slightly wounded, infections resulted in all cases. It would appear, then, that under the conditions of these experiments infection with *Phytomonas woodsii* does not occur in the absence of wounds.

The feeding punctures and injuries of such common carnation pests as red spider mites, thrips, and aphids under natural conditions doubtless

offer a means of entrance for the bacteria. In 2 experiments conducted in the greenhouse, carnations of the variety White Matchless, free from the bacterial spot but infested with red spider mites, were grouped around 3 plants heavily infected with the disease. All of the plants then were subjected to frequent overhead watering, with the result that infection was soon evident on the spider-infested plants. In another experiment, however, bacteria in a water suspension were painted upon spider infested leaves of White Matchless with negative results. It seems reasonable to assume, nevertheless, until further evidence is obtained, that under natural conditions the bacteria gain entrance chiefly through wounds.

The effect of air temperature on the rate of development of the bacterial-spot lesions (Fig. 1, C to F) has been studied in a series of 3 experiments. Vigorously growing plants of the varieties Sophelia and Laddie were used. Five inoculations were made on each of 3 leaves of 4 plants, making a total of 60 inoculations. These were accomplished by puncturing the epidermis of the leaves with a sterilized needle and then painting the wounds with a water suspension of the bacteria. The diameter of the lesions was measured 15 days after inoculation and the data from the 3 experiments were summarized (Fig. 2). Development of the lesions was relatively slow at 60° F., with a rapid increase at the higher temperatures to the optimum at 75° F.

THE PATHOGEN

In describing the pathogen, 3 isolates were used. One was isolated in September, 1931, from carnation leaves of the variety White Matchless, collected at Skaneateles, New York. A second was a similar isolate, but reisolated in March, 1932; and the third was isolated in July, 1932, from specimens of the variety Sophelia, collected on Long Island. All 3 isolates behaved similarly.

Following is a description of the pathogen in culture:

Phytomonas woodsii is a small rod with rounded ends or at times pointed, occurring singly or less commonly in pairs. Spores are not formed, but frequently a central volutin granule is observed. A culture 48 hours old on beef extract agar (pH 6.85) grown at 27° C. showed the following cellular measurements: 1.56 μ (1.05–2.10 μ) by .67 μ (.47–1.05 μ). A Congo red negative stain was used in this case. A direct stain no doubt would give smaller measurements.

The organism is motile, having one or more polar flagella; is Gram-negative, and stains readily with carbol fuchsin. It is aerobic.

On beef extract agar (pH 7) at 27° C., growth is slow and the colonies are small, circular, and colorless. On slants, growth is scant, filiform, and somewhat creamy by transmitted light. Its consistency is butyrous.

Growth on potato-dextrose agar is more abundant and glistening. Brom-cresol-purple milk becomes alkaline (bluish), but no other change takes place. There is slight growth in Uschinsky's solution and in Clara's solution (4), but there is no green fluorescence. No liquefaction occurs in gelatin. Light hydrogen sulphide production is found in lead acetate agar. Ammonia is produced in beef-extract bouillon. Hansen's method of determination was followed. Nitrates are not reduced to nitrites. The method used was that of sulfanilic acid and a naphthalamine in acetic acid. The pathogen, when grown in tryptophane broth and determination made at the end of 1 and 4 days with the Ehrlich-Böhm test, showed no indication of indol production.

The ability of the pathogen to ferment carbohydrates was determined by methods employed and described in a previous publication by one of the writers (3). In the synthetic medium used, growth was slow, as is true on most media. Growth with a change in the hydrogen-ion concentration of the medium occurred with the following carbohydrates: dextrose, levulose, galactose, arabinose, xylose, rhamnose, lactose, glycerol, mannitol, acetic acid, citric acid, malic acid, and succinic acid. No growth occurred in sucrose, maltose, salicin, cellulose, lactic acid, and formic acid. Starch is not hydrolyzed.

CONTROL

Experiments on the control of the disease have not yet been conducted by the writers, but certain observations in ranges where carnations are being grown commercially have indicated promising leads. Many New York State florists have obtained benefit from the application of sulphur dust, while Gregory (5) in Indiana reports control of the bacterial leaf spot with 2 applications of a zinc sulphate spray. In several cases rapid spread of the disease has been stopped by fumigating rather than syringing for the red-spider control. General sanitary measures and maintenance of the greenhouse temperature and humidity as low as possible in the early fall also are advisable.

SUMMARY

The bacterial leaf spot of the carnation caused by *Phytomonas woodsii* is described and its economic importance discussed. Observations are made on the varietal susceptibility of the carnation to the pathogen. The pathogenicity of the causal organism, especially its relation to temperature is treated and illustrated. A description of *Phyt. woodsii* and its biochemical reactions are given.

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COLLETOTRICHUM TRUNCATUM (SCHW.), N. COMB., ON GARDEN AND LIMA BEANS

C. F. Andrus and W. D. Moore

(Accepted for publication July 17, 1934)

Specimens of Lima-bean stems and pods, bearing what appeared on casual examination to be a species of Vermicularia, have been collected by the writers from various sources in the Southeastern United States since 1931. In addition to severe russeting or scarification, the pods and stems bear numerous black pycnidia-like bodies that, on closer examination, prove to be sporodochia of a typical Colletotrichum, with curved spores produced among numerous dark brown setae. In January, 1934, specimens on Lima-bean pods, together with a pure culture of the fungus, were received from T. D. Persons, of the State Plant Board of Mississippi, through Miss A. E. Jenkins, of the Division of Mycology and Disease Survey. Since this material was accompanied by a request for identification, the writers have sought to trace the history and affinities of the organism and have made such emendations to earlier descriptions as seemed desirable.

The fungus has been found identical with one described by Schweinitz in 1832 as *Vermicularia truncata* Schw. on stems of *Phaseolus* sp. in Pennsylvania. Schweinitz's diagnosis was as follows:

"Peritheciis lineam vix excedentibus, nigris, (in leguminibus majoribus), adnatis, confertis, hemisphaericis, horizontaliter truncatis in majoribus; in minoribus irregulariter conico-truncatis, rugulosis. Setis filiformibus praesertim truncaturam occupantibus, divergentibus, longioribus, brevioribus immixtis. Saepe setae caducae sunt, ita ut perithecium orbatur.

Hab. in caulibus, sarmentis ac leguminibus Phaseolorum horti frequens, Bethl."

An examination of a portion of the original Schweinitz specimen in the Michener Collection, now in the Mycological Collections of the Bureau of Plant Industry, showed that the host probably was a Lima bean. No spores were found in several mounts made from the limited amount of material available, and Schweinitz gave no spore description. The general appearance and dimensions of the sclerotial bodies ("perithecia"), however, conform to the senile sporodochia on the Lima-bean pods from Mississippi; and also the abundance, pigmentation, and dimensions of setae are quite identical to those on the Lima-bean pods and likewise to those on bean stems artificially inoculated with the fungus sent by Persons.

The same fungus was described by Cooke in 1883 as *Vermicularia polytricha* Cke., based on a specimen collected in South Carolina (Ravenel's

F. Amer. Exs. 532). Cooke's diagnosis is as follows:

"Erumpens, cupulaeformis, strigosa, atra. Setis dense congestis, erectis, rigidis, subulatis, glabris, atrofuscis. Sporis lanceolatis, lunatis, nucleatis, hyalinus, (.03 × .004 mm.).

On Lima bean, (Rav., 2988). S. Carolina."

A comparison of the original specimens described by Schweinitz and by Cooke, together with a comparison of the author's descriptions, leaves little doubt that the two collections are nearly or quite identical, and are identical also with the recent collection from Mississippi.

The same fungus was again described by Heald and Wolf in 1911 as *Colletotrichum caulicolum*. Their description was based on material from living stems of the common garden bean, *Phaseolus vulgaris*, from Texas. Their diagnosis is as follows:

"Acervulis sparsis, nigris, lenticularibus, 150–250 μ. Setis copiosis, brunneis, septatis, utrimque rotundatis vel superne acutis, 60–120 × 3.5–4 μ; basidiis 30–60 μ, cylindraceis, hyalinis, plerumque 1–2 septatis; conidiis falcatis, hyalinis, granulosis, 18–30 × 3.5–4 μ."

In supplementary remarks the fungus is said to be the cause of a destructive disease of the Kentucky Wonder bean, and, according to their description, the symptoms correspond to those produced on bean plants inoculated with the Mississippi culture. Unfortunately, no specimen of the original collection by Heald and Wolf is available for direct comparison, but there appears to be little reason to doubt that the fungus is identical with that described by Schweinitz and by Cooke, as well as with the Mississippi specimen.

As stated previously, the fungus is undoubtedly a true Colletotrichum with spores formed externally among numerous setae. The numerous dark sclerotial bodies suggest a pycnidial fungus, when observed without proper magnification, but no true pycnidia have been observed in any of the specimens.

In view of the above facts a new combination appears to be warranted and the following is proposed, together with a modified and amplified version of the description by Schweinitz:

Colletotrichum truncatum (Schw.), n. comb.

Vermicularia truncata Schw. Syn. Amer. bor. No. 1865 (Published 1832).

Vermicularia polytricha Cke. Grevillea 12:24. 1883–84.

Colletotrichum caulicolum Heald and Wolf. Mycologia 3:10. 1911.

Acervulis lineam vix excedentibus, nigris, (in leguminibus majoribus), adnatis, confertis, hemisphaericis, horizontaliter truncatis in majoribus; in minoribus irregulariter conico-truncatis, rugulosis; setis copiosis, variabilibus, filiformibus praesertim truncaturam occupantibus, divergentibus,

longioribus, brevioribus immixtis, 60–300 × 3.5–8 μ; saepe setae caducae sunt, ita ut sclerotium orbatur; conidiis falcatis, lanceolatis, hyalinis, 18–30 × 3–4 μ.

On stems and pods of *Phaseolus lunatus* and on stems of *P. vulgaris* in Southern and Eastern United States.

In addition to the material sent from Mississippi by Mr. Persons, the Mycological Collections of the Bureau of Plant Industry now include por-

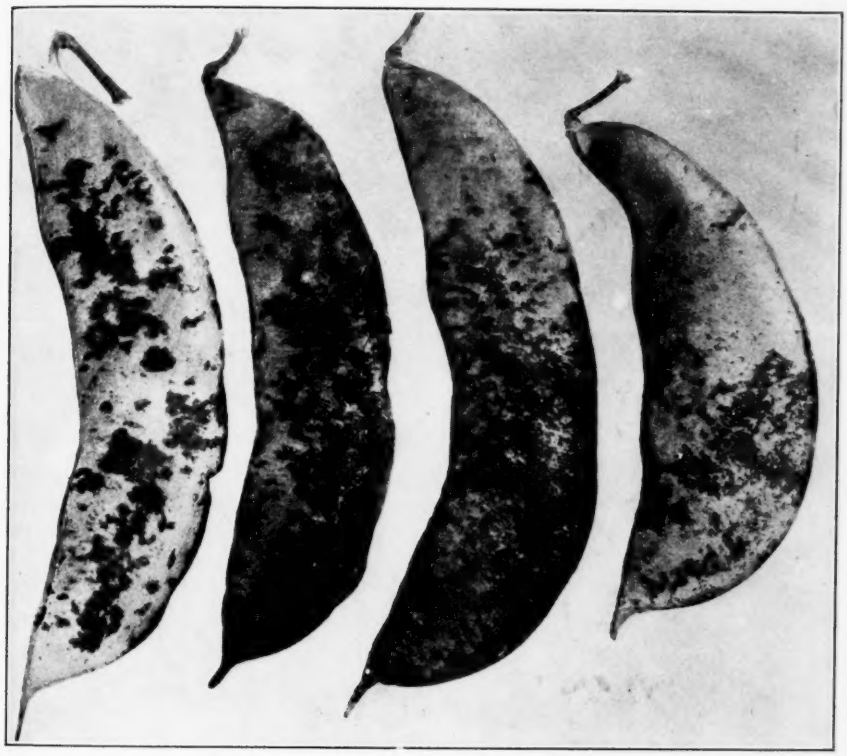

FIG. 1. Early stage of infection by *Colletotrichum truncatum* on Lima-bean pods.

tions of both the Schweinitz and Cooke specimens and also several collections that have been identified by various individuals as either *Vermicularia truncata* or *V. polytricha*.

The disease caused by this species of Colletotrichum, although not referred to in any known compendium of plant diseases, is by no means rare or local in distribution. Communications from pathologists in the Southern States indicate that it is not uncommon on Lima beans and is occasionally of some importance. In 1931 a field of 20 acres of Lima beans was found at Goldsboro, North Carolina, with about 5 per cent of the

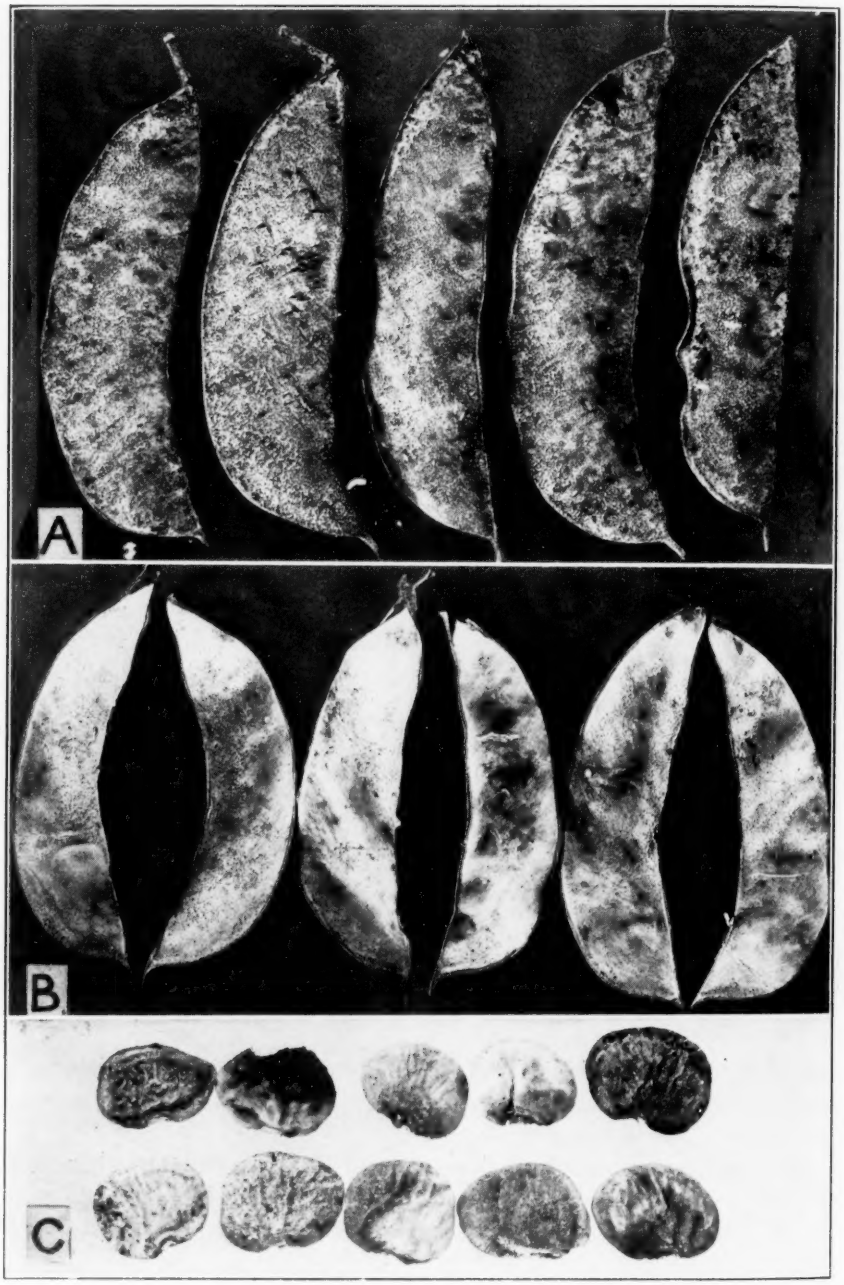

FIG. 2. A. Advanced stage of infection by *Colletotrichum truncatum* on Lima-bean pods, showing setose fruiting bodies superficially resembling pycnidia. B. Fruiting bodies on the inner side of pods. C. Type of injury on Lima-bean seed, with formation of sclerotial bodies on the surface.

plants heavily infected. During the same season 2 other infected fields were found at Norfolk, Virginia, and numerous specimens were collected in stores at Fort Gaines, Georgia. Collections from Pennsylvania, South Carolina, Georgia, Louisiana, Texas, and Mississippi attest to its fairly wide distribution in the Southern and Eastern United States.

The initial stages of infection are seen first on the pods as small reddish blotches (Fig. 1), which may spread over the entire surface. Definite lesions are not found. In later stages of development the infected areas become light brown or grayish, and numerous "perithecia" may be observed macroscopically (Fig. 2, A). The organism also infects the plant branches, girdling and killing them in much the same way as does pod spot (*Diaporthe phaseolorum*). In many cases the dry infected pods will show numerous "perithecia" on the interior of the shells (Fig. 2, B), and not infrequently the seed in such pods will likewise show these sclerotial bodies over the surface (Fig. 2, C). It appears, therefore, that the fungus may be spread through infected seed.

Natural occurrence of the fungus on garden beans is known only from the description by Heald and Wolf. Artificial infections, however, have been produced on 18 varieties of *Phaseolus vulgaris*,—the symptoms ranging from slight scarification of stems and leaves to very severe stem injury accompanied by defoliation. Artificial infections on varieties of *P. lunatus* indicate that the latter species is more completely susceptible than *P. vulgaris*.

Colletotrichum truncatum is entirely distinct from the common bean anthracnose organism, *C. lindemuthianum*. The characters in which it differs most conspicuously from *C. lindemuthianum* are the curved, lanceolate spores and habit of growth in culture. The types of injury produced by the 2 species on beans also differ conspicuously.

The authors wish to acknowledge the assistance given by Dr. W. W. Diehl, of the U. S. Department of Agriculture, in the examination of herbarium specimens and in the preparation of the manuscript.

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SOME EFFECTS OF PHYMATOTRICHUM ROOT ROT ON THE MICROSCOPIC CHARACTERS OF COTTON FIBERS[1]

G. N. Stroman, J. J. Taubenhaus, and Walter N. Ezekiel

(Accepted for publication April 26, 1934)

The cotton root rot, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, kills cotton plants at various times during the growing season. Plants attacked early in the season produce only occasional immature bolls, while those that succumb later may already have matured a large part of their crop. In separate papers, some of the writers have discussed the relative yields obtained from plants that succumbed to root rot at different times during the season,[2] and also the effect of the death of plants from attack by root rot at the various times on the quality of lint and seed produced.[3] The present paper reports the results of microscopic examination of fibers from these same cotton plants.

MATERIAL AND METHODS

The material studied consisted of thirteen lots of cotton lint from a single field of Startex cotton grown under irrigation at College Station, Texas, in 1931. Of these lots, 11 were from plants that had died or had shown advanced symptoms of Phymatotrichum root rot at various intervals during the season of 1931; while 2 lots were from plants that were still apparently normal on September 10, when the first picking was made. A random sample was taken from each lot of cotton for microscopic study of the fibers. From the sample a tuft of fibers was mounted in glycerine in parallel fashion on a microscope slide and a cover glass placed on top. Thirty individual fibers from each lot of cotton were measured as to ribbon width, thickness, and number of convolutions or natural twist. According to Pope,[4] more than 100 fibers should have been measured. This was not possible in the present work, but the results given below are treated so as to show their statistical significance.

The ribbon width is the measurement taken across the flat surface of the fiber. It was taken at the widest point or points of the fiber. The thick-

[1] Approved for publication by the Director of the New Mexico Agricultural Experiment Station and by the Director of the Texas Agricultural Experiment Station as Technical Contribution No. 296.

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TABLE 1.—*Means, standard deviations, and ranges in measurements of microscopic characters of cotton fibers, from plants that succumbed to root rot at various periods during the growing season. Averaged from measurements of 30 individual fibers, in each lot*

Lot No.	Interval during which plants succumbed to root rot	Width of fibers, in microns			Thickness of fibers, in microns			Twist, convolutions per 1.6 mm. of fibers		
		Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range
1	to June 30[a]	21.41	4.91	8.7–29.0	11.24	3.47	4.2–18.8	11.73	2.21	8–16
2	July 1 to July 7[b]	21.90	4.23	14.4–32.0	11.55	2.07	8.7–17.4	11.53	3.56	6–20
3	" 8 to " 15	26.74	4.34	20.3–36.4	14.87	3.62	8.7–21.8	8.93	2.91	5–17
4	" 16 to " 22	26.77	5.01	15.9–37.7	15.45	3.39	10.2–24.5	9.37	1.91	7–14
5	" 23 to " 30	24.54	4.59	15.9–33.4	13.48	3.66	7.2–21.8	10.50	3.42	7–16
6	" 31 to Aug. 4	23.38	3.34	17.4–29.0	13.14	2.62	8.7–18.8	10.43	2.51	6–16
7	Aug. 5 to " 11	22.03	3.60	14.4–29.0	12.34	2.50	8.7–17.4	14.60	2.94	10–20
8	" 12 to " 18	21.65	3.05	14.4–27.5	12.04	2.29	8.7–17.4	14.80	2.75	10–20
9	" 19 to " 25	20.82	3.55	11.4–29.0	11.85	2.35	8.7–20.3	14.03	3.41	10–22
10	" 26 to Sept. 3	20.84	3.00	14.4–29.0	11.57	2.35	7.2–15.9	13.03	4.14	5–20
11	Sept. 4 to " 10	21.41	3.66	14.4–30.5	12.02	3.22	7.2–20.3	15.20	4.16	7–25
12	Normal plants	21.16	3.55	12.9–29.0	11.51	2.35	5.7–15.9	13.27	2.63	9–22
13	Normal plants	23.02	3.13	18.8–29.0	11.95	2.26	8.7–17.4	14.13	2.78	7–18
	Average of normal plants	22.09			11.73			13.70		

[a] Only 8 bolls were borne by the 77 plants of this group; 2 of these bolls, yielding 1.9 gm. of the 6.6 gm. of lint cotton, were borne on plants that recovered and were still green on September 10.

[b] The single boll harvested from the 49 plants in this group was borne by a plant that recovered and was green on September 10.

ness was measured at small places on the fiber between two of the convolutions. However, due to the twisting of the fibers, this measurement probably was greater than the actual thickness of the fibers. The thickness and width of fibers were measured with a micrometer eye piece. The width and thickness were taken at 5 different places on each fiber and averaged. The convolutions or twists of the fiber were obtained by counting the number of twists along about 1.6 mm. of the cotton fiber, that is, across the low-power field of vision.

RESULTS

The mean, standard deviation, and range of the measurements of the fibers are summarized in table 1. In general, the fibers from plants that succumbed to root rot between July 8 and August 4 showed distinct differences in microscopic characters of fibers from plants that succumbed later in the season or from the normal plants. With lot 1, part of the crop, and with lot 2, all of the crop were from plants that had recovered from the disease, and the characters of the lint from these lots were, in general, similar to those of the normal plants.

Ribbon Width of Fiber

The mean width of fibers in lot 3 was $26.74 \pm .53\mu$, and of fibers from the normal plants of lot 12 was $21.16 \pm .44\mu$. The difference between these two lots was $5.58 \pm .69\mu$, a significant difference. The values for lot 4 were practically the same as for lot 3. The difference between the mean widths for lots 5 and 12 was $3.38 \pm .71\mu$. Similarly, lot 6 differed from lot 12 by a fairly significant figure of $2.22 \pm .60\mu$. The measurements for the two lots of normal plants, while not identical, were not greatly different; the difference amounting to $1.86 \pm .58\mu$, barely 3 times its probable error.

Thickness of Fiber

The fibers of lots 3, 4, and 5 were significantly thicker than those from the normal plants. Lots 3 and 13 differed by $2.92 \pm .53\mu$. As noted above, the thickness as measured was perhaps not precisely indicative of the actual thickness of fibers. The recorded thicknesses for lots 3, 4, and 5 were, therefore, possibly biased by the smaller number of twists in fibers of these lots.

Convolutions or Natural Twist of Fiber

Lots 3, 4, 5, and 6 were again distinct from the other lots in the frequency of twisting. They averaged fewer twists per unit length. Of these 4 lots, the one with the larger number of twists was lot 6, with $10.43 \pm .31$ twists per 1.6 mm. of fiber. Comparing this with lot 12, which

had fewer twists of the 2 lots of normal plants, 13.27 ± .32 twists, there is a difference of 2.84 ± .45 twists, over 6 times its probable error. In this character, lots 1 and 2 were intermediate between these lots mentioned (3, 4, 5, and 6) and the normal plants. Lot 2, with 11.73 ± .27 twists, differed from lot 12 by 1.54 ± .42 twists, a fairly significant difference, since it is 3.7 times its probable error.

DISCUSSION

In general, the cotton fibers from the lots of cotton that died early in the season (excluding lots 1 and 2) had microscopic characters significantly different from those of the normal cotton fibers. It may be deduced that these less twisted, coarser fibers from plants killed early in the season by root rot probably would have a lower spinning value than normally developed fibers and would be more likely to be discarded as waste in the spinning. These results explain the inferior quality and strength of yarns spun in some earlier work,[5] from lint of cotton plants killed early in the season by root rot, as compared to yarns from cotton from normal plants or from those attacked later in the season.

In the absence of some experimental evidence bearing on these relations, any attempt to explain the excess width and thickness of fibers from plants that succumb to root rot early in the summer must remain completely hypothetical. The abnormal characters of the fibers may result from the effects of translocation of soluble materials of some sort upward from the areas invaded by the fungus, prior to the final death of the plant; or of materials from the stem, which may remain green after the leaves are shed; or from environmental influences on the fibers in the bolls during the month intervening between death of the plants and picking.

It is of considerable importance that the apparently undesirable type of fiber did not constitute a very large proportion even of the material harvested from plants with root rot in the experimental work under consideration. As shown in detail in another paper of this series,[5] the greater losses in yield occurred with plants succumbing to root rot early in the season. Of the cotton picked from plants that had succumbed to root rot, slightly more than 90 per cent came from plants that had died in the 5 weeks immediately preceding harvest; that is, during the period in which the microscopic characters of the fibers were apparently not significantly affected by death of the plants from root rot. Less than 10 per cent of the yield from these root-rot plants in the College Station experiment was obtained from the plants that succumbed to the disease during the preceding 4 weeks, during which evident changes of the fibers apparently were associated with root-rot injury.

[5] Campbell, M. E., and H. H. Willis. Spinning test of root rot cotton (Texas—crop of 1927). U. S. Dept. Agr. Bur. Agr. Econ. 1928. (Mimeographed).

However, the continued irrigation in the experimental field encouraged continued spread of root rot during the season. Under dry-farming conditions, during many seasons,[5] the spread is, instead, lessened with the advent of dry summer weather. Therefore, of the plants with root rot, a higher proportion succumb earlier in the season, during the period when perceptible injury to the quality occurs. It follows that under dry-farming conditions more than 10 per cent, although probably less than 20 per cent, of inferior fibers might be expected in the cotton picked from plants that succumb to root rot. Thus, in a field in which as much as half of the crop came from plants that had succumbed to root rot, 5 to 10 per cent of the crop might consist of such inferior fibers; while if only 10 per cent of the crop had been harvested from plants with root rot, no more than 1 or 2 per cent of inferior fibers would be expected. In the exceptional cases in which the majority of cotton plants in a field have succumbed to root rot early in the season, it is obvious that the small crop that could be harvested would consist largely of the inferior fibers.

SUMMARY

Microscopic studies have been made of cotton fibers from plants killed by root rot at different periods between June 30 and September 10, 1931. In each sample, 30 fibers were measured as to ribbon width, thickness, and number of convolutions per unit of length. The mean, standard deviation, and range of individual fibers in each lot are tabulated. Fibers from plants killed early in the season were wider and thicker and had a smaller number of convolutions per unit length than fibers from normal plants. Fibers from plants killed later in the season were not significantly different from those from the normal plants.

It is pointed out that the coarser, less twisted fibers from plants succumbing early in the season constituted less than 10 per cent of the cotton harvested from the root-rot plants grown under irrigation in this experiment, and would probably average not more than 20 per cent of the crop from plants that succumb to root rot under dry-farming conditions. (New Mexico and Texas Agricultural Experiment Stations, cooperating. Agricultural Experiment Station, Las Cruces, N. Mex., and College Station, Texas).

[6] *Loc. cit.*